FINAL REPORT

Enhancing Natural Attenuation through Bioaugmentation with Aerobic Bacteria that Degrade *cis*-1,2-Dichloroethene

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Executive Summary

Monitored natural attenuation (MNA) and enhanced in situ bioremediation (EISB) remedies hold the promise of reducing the costs associated with the cleanup of Department of Defense (DoD) sites impacted by chlorinated solvents. However, there are many DoD sites where tetrachloroethene (PCE) and trichloroethene (TCE) are undergoing only partial dechlorination to *cis*-1,2-dichloroethene (cDCE), even when sufficient electron donor is present either because of the absence of required bacteria (*Dehalococcoides*) or aerobic conditions.

Under SERDP sponsorship (ER-1168), a novel aerobic bacterium (*Polaromonas* sp. strain JS666) that uses cDCE as a sole carbon and energy source was isolated and characterized (Coleman *et al.*, 2002a,b). Since it requires no exotic growth factors, JS666 is a promising bioaugmentation culture for aerobic sites where cDCE is recalcitrant. The microorganism will grow and thrive where oxygen and cDCE are co-located, and JS666 also degrades 1,2-dichloroethane (DCA) and cometabolizes TCE and vinyl chloride (VC). Ideal groundwater conditions for JS666 include: dissolved oxygen (DO) levels between 0.01 mg/L and 8 mg/L; low ionic strength (conductivity <15 milliSiemens per centimeter [mS/cm]); a pH of 6.5 to 8; and relatively low concentrations of TCE, 1,2-DCA and VC (<500 µg/L).

The goal of this first field demonstration was to evaluate the effectiveness of JS666 in biodegrading cDCE. The demonstration was conducted at Site 21, St. Julien's Creek Annex in Chesapeake, Virginia. This site had several relatively well-characterized groundwater plumes of chlorinated volatile organic compounds (VOCs; primarily cDCE, TCE and VC), appropriate site conditions, and a suitable on-site support network. In the vicinity of the pilot test area, groundwater flow is towards the west. Shallow groundwater typically ranges from 2 to 7 ft below ground surface (bgs). Estimates of the hydraulic gradient and groundwater velocity for the Columbia aquifer are 0.004-0.01 ft/ft and 72 ft/yr, respectively (CH2M HILL, 2008). Preliminary baseline sampling indicated that the groundwater pH was in the 6 to 6.3 range and that buffering would be required.

For this demonstration, the site was instrumented to create four test plots within the pilot test area: a bioaugmentation plot receiving JS666, oxygen, and buffer (Plot #1); a bioaugmentation plot receiving JS666 and buffer (Plot #2); a control plot receiving buffer (Plot #3); and a control plot receiving oxygen and buffer (Plot #4). The intent of the two bioaugmentation plots was to establish the effect of adding JS666 and additional oxygen on the rate of biodegradation, while the corresponding control plots were intended to account for the effects of buffer and buffer and oxygen on the results in the bioaugmentation plots. Two upgradient wells (MW-11 and MW-7) served as background controls to monitor the groundwater in the absence of amendments.

The monitoring network for each of the bioaugmentation plots consisted of one fully screened injection well and 7 fully screened monitoring wells (one well upgradient of the injection well, 2 wells transgradient to the injection well, and 4 wells downgradient of the injection well). The control plots comprised a smaller well network of one fully screened injection well and 2 fully screened downgradient monitoring wells, located upgradient and transgradient to the bioaugmentation plots

Two down-well Waterloo Emitters in series were deployed in the injection well in Bioaugmentation Plot #1 and in Control Plot #4 to promote aerobic conditions. Each series of emitters was provided with a source of compressed air (rather than oxygen) because JS666 is sensitive to oxygen levels greater than 10 mg/L.

Field activities following well installation consisted of baseline sampling, buffer injections, aeration via down-well emitters (for Plots #1 and #4), and bioaugmentation (for Plots #1 and #2). Two bioaugmentations were performed during the demonstration: one in October 2008 and one in February 2009. During both bioaugmentations, approximately 8-9 L of culture (density of 10^8 colony forming units [cfu]/mL based on qPCR measurements) were injected into each bioaugmentation plot. The monthly field events consisted of groundwater sampling and buffer injections using extracted groundwater, with the exception of the final field event (May 2009) where only groundwater sampling was conducted.

Baseline sampling showed that the TCE levels ranged from 460 to 1200 ug/L in Plot #1, 180 to 320 ug/L in Plot #2, 230 to 620 ug/L in Plot #3, and 8 to 760 ug/L in Plot #4. cDCE concentrations ranged from 760 to 2800 ug/L in Plot #1, 560 to 990 ug/L in Plot #2, 100 to 310 ug/L in Plot #3, and 42 to 820 ug/L in Plot #4. VC concentrations were low in all plots ranging from 2.3 to 31 ug/L. Across all the plots, dissolved oxygen levels and oxidation reduction potentials ranged from 0.3 to 1.2 mg/L and 35 to -363 mV, respectively.

Following buffer addition and bioaugmentation, the increased pH and specific conductivity levels were generally sustained throughout the project duration as a result of continued buffer injections for all the plots. No significant changes in pH were observed in the upgradient wells in either bioaugmentation plot. In Plots #1 and #4, groundwater ORP and DO concentrations increased significantly in injection wells IW-01 and IW-04 (which were both equipped with emitters) throughout most of the demonstration.

Increases in alkalinity were predominately observed in wells immediately downgradient of the injection wells, with smaller increases in the transgradient wells. No significant change in alkalinity was observed in the upgradient wells for either bioaugmentation plot, indicating that downgradient increases were attributed to microbial activity stimulated by buffer addition and/or JS666 bioaugmentation.

Carbon stable isotope analysis supported a significant degree of cDCE biodegradation in many downgradient wells in Bioaugmentation Plots #1 and #2. In contrast, the main control on cDCE concentrations in the control plots was not biodegradation but fluctuations due to pumping and/or groundwater transport processes. Despite higher TCE concentrations in Plot #1, isotopic analyses indicated that more biodegradation occurred in Plot #1 (which received an oxygen emitter) versus Plot #2 (which did not). VOC analyses showed greater decreases in cDCE in many of the wells in the bioaugmented plots versus the control plots. However, the degree of biodegradation in Plot #1 was masked by the almost 2-fold increase in cDCE flowing into the plot during the course of the study.

Following bioaugmentation, qPCR and microcosm results demonstrated in-situ survival and activity of JS666 over the course of the demonstration in the bioaugmentation plots. Though the levels of JS666 were low (i.e., 3 x 10^3 to 10^4 CFU/mL), they were adequate to effect cDCE degradation, if suitable environmental conditions (adequate oxygen, pH and absence of inhibitory levels of TCE) were present. In general, there were very few qPCR detections in the control plots where no JS666 was added. Likewise there were no qPCR detections in either of the upgradient wells (MW-7 and MW-11), except for one instance of a 3.3 x 10^3 CFU/mL detection in MW-11. Thus, the pilot tests were successful in demonstrating the spread and stability of the JS666 organisms in the bioaugmented plots.

The microcosms were apparently more sensitive detectors of JS666 than was qPCR – i.e., positive microcosm activity in downgradient samples was observed in many instances where qPCR was negative. It should be noted that microcosm assays were conducted at 22^{o}C (compared to 17^{o}C of groundwater) and were not oxygen-limited. On the other hand, field D.O. levels were quite low. These results demonstrated that the JS666 maintained their potential for cDCE degradation, even when field conditions precluded activity.

The ease of use of this technology was evaluated based on our experience in the field with the bacteria. Addition of the culture via injection wells was straightforward because it was an aerobic culture. Therefore, no special procedures were required to exclude oxygen during the injection. Because the native groundwater pH was low at this site, buffering was required. To distribute the buffer throughout the injection area, groundwater was extracted, amended with buffer, and then reinjected. Although the procedure was simple, it was time-consuming and needed to be repeated due to the soluble nature of the buffer employed. Aeration using the Waterloo Emitter was easy (only requiring change out of the compressed air cylinder approximately monthly) but was not effective in distributing oxygen beyond the injection well. JS666 should be employed in aquifers with detectable dissolved oxygen or perhaps in an active recirculation system where oxygen can be metered into the injection stream continually.

This bioaugmentation technology was compared to pump and treat over a 30 year time period. The cost analysis shows a projected cost savings of 47%, assuming no aeration or buffering is required and sufficient oxygen is present in the groundwater naturally. Thus, under these assumptions, this technology is more cost-effective than pump and treat.

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LIST OF ACRONYMS

°C	Degrees Celsius
1,2-DCA	1,2-dichloroethane
AFB	Air Force Base
bp	base pair
cDCE	*cis*-1,2-dichloroethene
cells/mL	Cells per milliliter
cfu	Colony forming units
CMO	cyclohexanone monooxygenase
CO_2	carbon dioxide
COC	Chain-of-custody
CSIA	Compound specific isotope analysis
dH_2O	Distilled water
DHGs	Dissolved hydrocarbon gases
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DoD	Department of Defense
DOT	Department of Transportation
EISB	Enhanced in situ bioremediation
EPA	Environmental Protection Agency
ESTCP	Environmental Security Technology Certification Program
FRTR	Federal Remediation Technologies Roundtable
ft	Feet
ft bgs	Feet below ground surface
ft/yr	Feet per year
g	Grams
GC	Gas chromatograph
GIT	Georgia Institute of Technology
gpm	Gallons per minute
HCl	Hydrochloric acid
HDPE	High-density polyethylene
HSA	Hollow stem augers
IC	Ion chromatography
ID	Identifier
ISE	Ion-selective electrode
ISO	isocitrate lyase
KH_2PO_4	potassium monobasic orthophosphate
K_2HPO_4	potassium dibasic orthophosphate
L	Liter
Mb	Mega base pairs
MCL	Maximum contaminant level
mg/L	Milligrams per liter
mL	Milliliters
mM	Millimolar
MNA	Monitored natural attenuation

mS/cm	MilliSiemens per centimeter
MSDS	Material safety data sheet
MS/MSD	Matrix spike/matrix spike duplicate
MSM	Minimal Salts Medium
mV	Millivolts
NaOH	Sodium Hydroxide
nM	Nanomolar
nmol/min/mg	Nanomoles per minute per milligram
OD_{600}	Optical Density at 600 nm
O&M	Operation and Maintenance
ORP	Oxidation-reduction potential
P&T	Pump-and-treat
PCE	Tetrachloroethene
PTA	Pilot test area
PVC	Polyvinyl chloride
QA/QC	Quality assurance/quality control
qPCR	Quantitative polymerase chain reaction
RL	Reporting limit
RPM	Revolutions per minute
rRNA	Ribosomal Ribonucleic Acid
SERDP	Strategic Environmental Research and Development Program
SIC	Standard Industrial Classification
SJCA	St. Julien's Creek Annex
SRS	Savannah River Site
TCE	Trichloroethene
tDCE	*trans*-1,2-dichloroethene
UIC	Underground injection control
UNI	Universal
USEPA	United States Environmental Protection Agency
VC	Vinyl chloride
VOA	Volatile organic analysis
VOCs	Volatile organic compounds
µg/L	Micrograms per liter
µL	Microliter
µM	Micromolar
µmol	Micromole

1.0 INTRODUCTION

1.1 BACKGROUND

Monitored natural attenuation (MNA) and enhanced in situ bioremediation (EISB) remedies hold the promise of reducing the costs associated with cleanup of Department of Defense (DoD) sites impacted by chlorinated solvents. However, there are many DoD sites where tetrachloroethene (PCE) and trichloroethene (TCE) are undergoing only partial dechlorination to *cis*-1,2-dichloroethene (cDCE), even when sufficient electron donor is present/added. *Dehalobacter, Desulfitobacterium, Dehalospirillum, Desulfomonile, Desulfuromonas, and Enterobacter* are found widely in the environment, and can dechlorinate PCE and TCE to cDCE, but are incapable of further dechlorinating cDCE to vinyl chloride (VC) or ethene (Geosyntec, 2005). As a result, there are a significant number of plumes at DoD and related sites where PCE and TCE have been dechlorinated to cDCE, but where the cDCE persists and migrates uncontrolled in groundwater rather than undergoing further dechlorination to ethene (the desired end product in MNA and ESIB remedies).

Dehalococcoides are the only known group of microorganisms that can dechlorinate cDCE via VC to ethene. While *Dehalococcoides* are present at many sites, they are not ubiquitous in the environment (Hendrickson *et al*., 2002). Furthermore, anaerobic bioremediation/bioaugmentation may not be the best remediation strategy at sites with large cDCE plumes in aerobic aquifers. Instead, aerobic biotreatment of the cDCE may be more cost-effective, provided that this process can be induced to occur over the target treatment area.

Until recently, aerobic biodegradation of cDCE was thought to occur cometabolically, requiring the addition of an appropriate primary substrate, such as methane, propane, or toluene, to stimulate the co-oxidation of cDCE, and these processes were generally determined to have limited feasibility for large-scale field application. However, recent research conducted under SERDP sponsorship (ER-1168) has isolated and described a novel aerobic bacterium (*Polaromonas* sp. strain JS666) that uses cDCE as sole carbon and energy source (Coleman *et al.*, 2002a,b). Since it requires no exotic growth factors, JS666 is a promising bioaugmentation culture for aerobic sites where cDCE is recalcitrant. In essence, this microorganism can be used to achieve MNA without any further intervention other than adding it to groundwater because the microorganism will grow and thrive where oxygen and cDCE are co-located.

1.2 OBJECTIVES OF THE DEMONSTRATION

The goal of this field demonstration was to evaluate the effectiveness and robustness of JS666 as a bioaugmentation culture to enhance the biodegradation of cDCE. No field demonstrations of this technology have been conducted to date. The demonstration described herein represents the first demonstration of the effectiveness of JS666 for degrading cDCE in the field.

The objectives of the field demonstration were to:

1. Assess JS666's ability to degrade cDCE and other chlorinated ethenes/ethanes in-situ;

2. Evaluate the ability of JS666 to compete with indigenous microorganisms;

3. Evaluate the use of molecular markers to detect the spread of JS666 in groundwater;

4. Evaluate the effectiveness of isotopes to detect and quantify cDCE biodegradation; and

5. Provide reliable technical data relevant to field-scale aerobic biotreatment using JS666, including documenting benefits of the technology in terms of expected reduction in the duration and cost of remediation of sites where cDCE persists in groundwater.

1.3 REGULATORY DRIVERS

The United States Environmental Protection Agency (USEPA) maximum contaminant level (MCL) for cDCE in drinking water is 70 micrograms per liter (µg/L), 5 ug/L for TCE, and 2 ug/L for VC. While several sites have observed successful dechlorination of PCE and/or TCE plumes to ethene, there are a significant number of DoD and related sites where PCE and/or TCE plumes have been dechlorinated to cDCE, but where the cDCE persists and migrates uncontrolled in groundwater rather than undergoing further dechlorination to ethene. Groundwater cDCE concentrations at these sites can be considerably higher than the USEPA MCL. The JS666 technology strives to reduce cDCE concentrations below the MCL.

2.0 TECHNOLOGY

The following sections provide a description of the technology (Section 2.1); discuss the technology development (Section 2.2); and outline the advantages and limitations of the technology (Section 2.3).

2.1 TECHNOLOGY DESCRIPTION

2.1.1 Characteristics of *Polaromonas* sp. strain JS666

Through research conducted under SERDP sponsorship (ER-1168), a novel aerobic bacterium (JS666) was isolated that is able to use cDCE as the sole carbon and energy source under aerobic conditions. It converts cDCE to carbon dioxide and water without the addition of exotic co-factors (Coleman *et al.*, 2002a,b). This organism was found in only one of 37 samples screened for ability to aerobically oxidize cDCE. Thus, while not necessarily unique, it appears to be relatively rare. Since it requires no exotic growth factors, JS666 is a promising bioaugmentation culture for aerobic sites where cDCE is recalcitrant. In essence, this microorganism can be used to achieve MNA without any further intervention other than adding it to groundwater because the microorganism will grow and thrive when oxygen and cDCE are co-located. Though cDCE and 1,2-DCA are the only known solvents (thus far) to serve as growth substrates for JS666, this microorganism can co-metabolize several other chloroethenes (TCE, *trans*-1,2-dichloroethene [tDCE], and VC) while growing on cDCE.

The 16S ribosomal deoxyribonucleic acid (DNA) sequence of strain JS666 has 97.9% identity to the sequence from *Polaromonas vacuolata*, indicating that the isolate is a β-proteobacterium. At 20 degrees Celsius (°C), strain JS666 grows on cDCE with a minimum doubling time of 73 ± 7 hours and a growth yield of 6.1 grams (g) of protein per mol of cDCE at its optimum pH of 7.2. The half-velocity constant (K_s) for cDCE transformation is 1.6 ± 0.2 micromolar (μM) and the maximum specific substrate utilization rate (k) ranges from 12.6 to 16.8 nanomoles of cDCE per minute per milligram of protein (nmol-cDCE/min/mg-protein) (Coleman *et al.*, 2002a). Most importantly, cDCE is degraded routinely to concentrations below 0.03 μg/L (Coleman *et al.*, 2002a).

In the laboratory phase of study, the relative kinetics and mutual effects of binary mixtures of cDCE at ~2 milligrams per liter (mg/L) in the presence of lesser concentrations (50 to 450 μg/L) of VC, TCE, or 1,2-DCA were investigated. Although the co-presence of VC, TCE, or 1,2-DCA reduced the maximum degradation rate of cDCE, the rate remained substantial and cDCE could be completely degraded, as could the co-substrates. Co-presence of VC or TCE caused cDCE degradation rates to be halved, but the effect was not proportional to concentrations of VC or TCE. On the other hand, degradation of the co-substrate was either improved (VC) or unaffected (TCE) by the presence of cDCE (Geosyntec, GIT & Cornell University, 2008).

The patterns of 1,2-DCA degradation in the presence of cDCE were different than those observed with VC and TCE. Clearer signs of true competition were observed with cDCE degradation in the presence of 1,2-DCA. cDCE was modestly inhibited by 1,2-DCA in a roughly linear decline with increasing 1,2-DCA concentration to 0.6 mg/L, and 1,2-DCA degradation was markedly inhibited by the much higher, 1.8 mg/L cDCE concentration. These results were consistent with the observation that JS666 can grow on 1,2-DCA, but not on VC or TCE (Geosyntec, GIT & Cornell University, 2008).

During laboratory studies, no evidence was found to suggest that the ability to degrade cDCE can be transferred from JS666 to indigenous bacteria. Therefore, it is necessary to ensure site conditions are suitable for the JS666 strain so that it can grow and thrive (Geosyntec, GIT & Cornell University, 2008).

2.1.2 Expected Applications of the Technology

JS666 can be incorporated into passive, active or semi-passive bioremediation systems or it can be injected once into groundwater with appropriate conditions to facilitate natural attenuation (otherwise known as enhanced attenuation).

Ideal conditions for JS666 include:

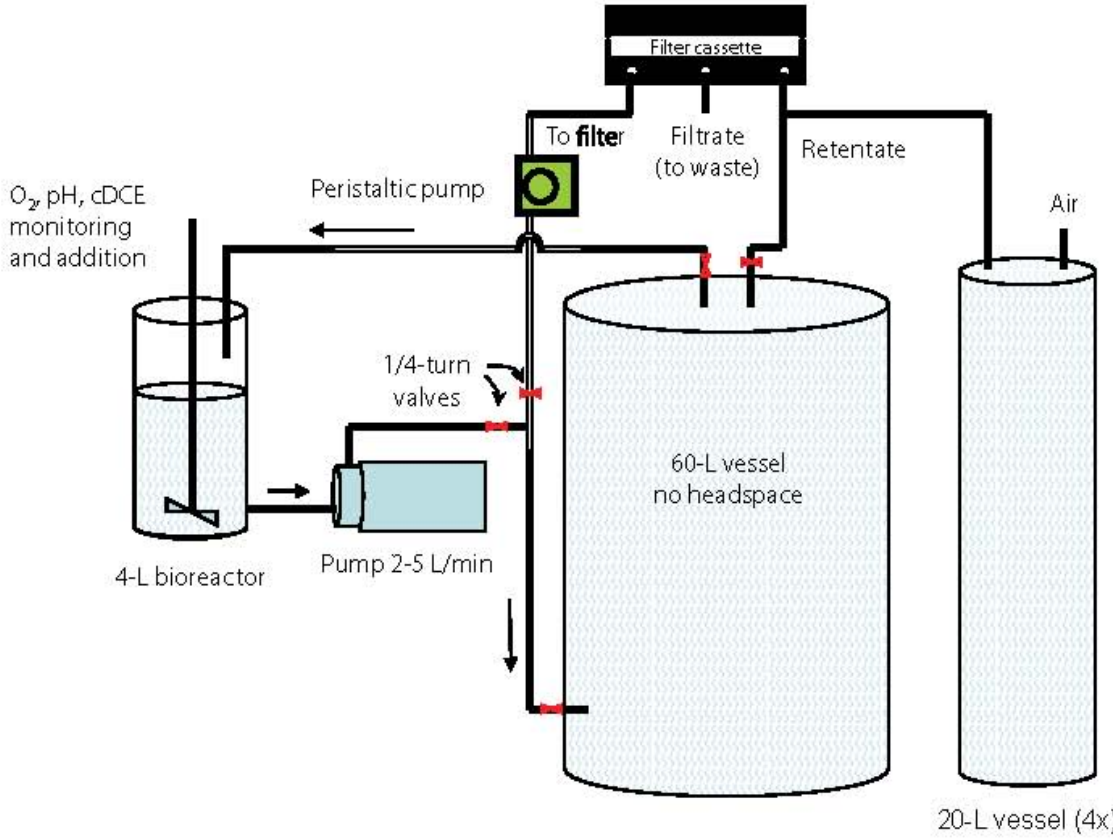
- Groundwater dissolved oxygen (DO) levels as low as 0.01 mg/L and as high as 8 mg/L;
- Groundwater with low ionic strength (conductivity <15 milliSiemens per centimeter [mS/cm]);
- Groundwater pH of 6.5 to 8; and
- Relatively low concentrations of TCE, 1,2-DCA and VC (<500 μg/L) in groundwater.

2.2 TECHNOLOGY DEVELOPMENT

2.2.1 JS666 Growth

A variety of laboratory experiments were conducted to establish factors that allow optimal cell growth for production purposes. Results of these experiments indicated that the JS666 culture could be effectively grown for field application. In addition, cells stored or stockpiled over a short period of time rapidly recovered the ability to degrade cDCE (Geosyntec, GIT & Cornell University, 2008).

A reactor system for growing 64-liter (L) batches of cells was designed as shown in Figure 2-1. The 4-L reactor served as a module for monitoring and adjusting cDCE, pH, O_2 and OD. A



Schematic of Bioreactors Used to Grow JS666
Site 21, St. Julien's Creek Annex, Chesapeake, VA

Geosyntec consultants

Guelph | October 2009

Figure **2-1**

centrifugal pump capable of flow rates up to 5 L/min circulated the culture from the 4-L reactor into a 60-L tank. The larger tank was filled without a headspace so that the overflow returned to the 4-L reactor. JS666 was grown in phosphate buffer (10 millimolar [mM], pH 7, 20°C) containing half-strength nutrients from Stanier's minimal salts base (Stanier *et al.*, 1966) with cyclohexanone (5 mM) as the carbon source. The slow growth on and high cost of pure cDCE dictated that the bulk of the growth be done on an alternative substrate such as cyclohexanone, which did not inhibit subsequent induction of cDCE degradation. When the OD_{600} reached 1.0, the growth substrate was changed to a mixture of 5 parts acetonitrile and 1 part cDCE (delivered by syringe pump to maintain 100 μM cDCE in the reactor) to allow induction on cDCE. After 2 days, the substrate was changed to pure cDCE and the culture was maintained on pure cDCE delivered by syringe pump to maintain a theoretical concentration of 100-1000 μM cDCE in the reactor until final harvest. Cultures were harvested by transverse flow filtration and the concentrated cells were either frozen at -80°C or diluted with cold (4°C) minimal medium to a total volume of 18-L for transport to the site.

2.2.2 Microcosm Studies

Microcosms were constructed with subsurface materials from five sites: Savannah River (SRS), Hill AFB, Robins AFB, Fort Lewis, and Aerojet. In neutral-pH-buffered microcosms constructed from all five site-materials, high concentrations (~ 60 mg/L) of cDCE were completely degraded within 10 to 15 days when inoculated with JS666 culture at 4×10^5 cells/mL. Without inoculation, no significant cDCE degradation was observed. Studies were undertaken to determine effective inoculum density, using three levels: 4×10^5 cells/mL, 4×10^4 cells/mL, and 4×10^3 cells/mL. In microcosms constructed with SRS soil and minimal salts medium (MSM), cDCE was depleted in about 20 days at 4×10^5 cells/mL and was about 50% depleted in 60 days at both 4×10^4 cells/mL, and 4×10^3 cells/mL inoculum levels. With a more realistic initial cDCE concentration (0.6 mg/L), complete degradation was observed in about 5 days at 4×10^5 cells/mL and 4×10^4 cells/mL, and in about 20 days at the 4×10^3 cells/mL inoculum level. Therefore, a minimum of 10^4 cells/mL is the suggested inoculum level for field application. All of these microcosm studies suggested that JS666 would survive and remain active in subsurface environments (Geosyntec, GIT & Cornell University, 2008).

2.2.3 Molecular Probe Development

To track the distribution and growth of JS666 in the field, two DNA-based probes were developed at Cornell University: 1) ISO (based on the isocitrate lyase gene of JS666); and 2) CMO (based on the cyclohexanone monooxygenase gene of JS666). Additionally, a putative universal probe (UNI) was employed that was intended to target the 16S rRNA gene of eubacteria. ISO and CMO were intended to be JS666-specific, while UNI was intended to capture most eubacteria and could thus serve as a "normalizer" if necessary. The concern going into this field study was that variable amounts of fines in well samples might result in a variable relationship between copy-numbers enumerated by JS666 probes and actual subsurface levels of JS666. Use of the UNI probe would allow, if later desired, the reporting of JS666 copy-numbers

normalized to UNI numbers (i.e., total eubacterial 16S rRNA numbers). Also, the inclusion of UNI counts would serve as a cross-check on extraction and qPCR steps (i.e., if ISO or CMO numbers were below detection the finding of "normal" UNI concentrations would indicate that low levels of ISO and CMO were not due to a bad extraction or a faulty qPCR run).

The ISO probe was based on the chromosomal gene, isocitrate lyase, of JS666. It seemed a suitable target for a molecular probe as it has ample variability and sequence stability for the design of strain specific primers. Additionally, it has more sequence variability than the 16S rRNA gene. Isocitrate lyase is a functional gene in the glyoxylate cycle. The Beacon Designer 4 software program aided in the design of the following isocitrate lyase primer set:

AceA 276F (TGCCGCTGACAACAACAC)

AceA 414R (ATCAATGCCTTTGGAGTGC)

The ISO probe was used in microcosms constructed with soil and groundwater from five field sites. Preliminary results revealed a strong correlation between the presence of JS666 and degradation of cDCE, suggesting the probe would be a useful tool for tracking JS666 movement in subsurface environments (Geosyntec, GIT & Cornell University, 2008).

To provide a normalization parameter for field studies, a technique to quantitatively measure total eubacterial 16S rRNA targets (UNI probe) was employed (Bach *et al.*, 2002), the 16S rRNA primers used,

799F (GGTAGTCYAYGCMSTAAACG) and

1044R (GACARCCATGCASCACCTG,

have a similar annealing temperature to that of the isocitrate lyase primers and were therefore run with the same protocol.

When early field results indicated that the ISO probe was not absolutely specific to JS666 (i.e., some positive results were occasionally observed in control wells), a second JS666-specific probe, cyclohexanone monooxygenase (CMO) was developed. Selection of CMO as a target was based on ongoing work on the elucidation of the metabolic pathways in JS666 completed through an integrated omics approach, which was used to identify genes that are up-regulated by cDCE versus the alternate reference substrate glycolate (Jennings *et al.*, 2009). One of these up-regulated genes encodes for a putative CMO protein and was chosen as an additional probing target. It is postulated that the function of CMO in JS666 is to catalyze DCE epoxidation.

Primers for the JS666 putative CMO gene were chosen as follows:

Cmo946F: ATTGTCAAAGACCCGGAAACTGCC,

Cmo1037R: TAAATGGCGTAGTAGCCGCTGTCA

The probes were designed using the PrimerQuest software available at the IDT website (http://scitools.idtdna.com/Primerquest/). Primer specificity was checked by BLAST analysis. Additionally, a melt curve was completed following the amplification reactions to confirm the specificity of the primers and the reactions. Moreover, the primers have a similar annealing temperature to those of the isocitrate lyase and universal eubacterial primer-sets and were, therefore, run with the same protocol.

2.3 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

Groundwater remediation approaches for VOC-impacted sites have historically employed groundwater extraction and ex situ treatment (i.e., pump-and-treat [P&T]). Unfortunately, these approaches have been largely ineffective in significantly improving groundwater quality, even after decades of continuous operation (National Research Council, 1994). As a result, remediation technologies such as MNA and EISB have received significant attention, because they are less intrusive, more effective, and less costly.

The main advantages of aerobic biotreatment using JS666 over other treatment technologies include:

- Potential for lower overall costs than alternative technologies such as groundwater P&T that have high operation and maintenance (O&M) costs;
- Potential for achieving cDCE biodegradation without any further intervention other than adding JS666 to groundwater (i.e., JS666 does not require exotic co-factors to survive); and
- cDCE (and potentially other VOCs) will be destroyed rather than transferred to another medium.

The main limitations of aerobic biotreatment using JS666 are:

- The presence of co-contaminants (e.g., TCE and VC) at concentrations that may be inhibitory to bioremediation by the JS666 culture;
- Aerobic groundwater with a near-neutral pH is required for optimal growth and activity of the JS666 culture; and
- Low pH groundwater requires the addition of buffer, which can be time-consuming.

3.0 PERFORMANCE OBJECTIVES

The performance objectives are provided in Table 3-1. Each objective is discussed in the following sections.

3.1 REDUCTION IN cDCE CONCENTRATIONS

3.1.1 Qualitative

A key performance objective was to obtain greater reductions in cDCE concentrations in the bioaugmentation plots than in the control plots so that the effect of the JS666 bacteria (rather than the addition of buffer and/or oxygen) could be assessed.

To evaluate this objective, groundwater samples from each of the plots were analyzed by EPA Method 8260, and data from bioaugmentation plots were graphically compared to data from control plots and from background (upgradient) wells over the course of the demonstration. Isotopic analyses were also used to identify greater cDCE biodegradation (through enrichment in $\delta^{13}C$) in the bioaugmentation plots versus the control plots.

The result was enhanced cDCE degradation in many (e.g., MW-1, MW-2, MW-3, MW-5, MW-6, MW-10, MW-12 and MW-14) but not all, of the bioaugmentation plot wells. Some wells did not exhibit significant cDCE degradation due to either TCE inhibition and/or oxygen limitations. Therefore, the reduction in cDCE concentrations objective was met for many but not all of the bioaugmented wells.

3.1.2 Quantitative

When cDCE concentration reductions in groundwater were quantitatively evaluated, the objectives were to achieve greater than 75% reduction in bioaugmentation plots over background concentrations and twice the reduction of cDCE concentrations in bioaugmented plots versus control plots. The reduction was evaluated by plotting normalized concentrations in each well (relative to baseline concentrations) for both bioaugmentation plots and control plots for the April 2009 sampling event. Although there were substantial cDCE declines in some of the bioaugmented wells (e.g., MW-2, MW-3, MW-10 in Plot #1 and MW-5, MW-6, and MW-14 wells in Plot #2), the % reduction was less than 75% relative to baseline concentrations and the reduction in the bioaugmented plots was not twice that of the control plots, likely due to TCE inhibition and/or oxygen limitation. Therefore, neither of these performance objectives was met.

3.2 GROWTH AND SPATIAL DISTRIBUTION OF JS666

The qualitative objective associated with the growth and distribution of JS666 was to observe the movement of JS666 away from the injection well. Achieving this objective is important so that the culture can be distributed throughout the treatment area. The further the

TABLE 3-1: Performance Objectives
Site 21, St. Julien's Creek Annex, Chesapeake, VA

Type of Performance Objective	Primary Performance Criteria	Expected Performance	Actual Performance Objective Met? *(to be completed following demonstration)*
Qualitative	1) Reduce cDCE concentrations	Greater reduction of cDCE concentrations in bioaugmented plots than in control plots	Yes, in some wells
	2) Spread and growth of JS666	Growth and spatial distribution of JS666 away from injection wells. Higher numbers of JS666 in bioaugmented plots than in control plots	Yes
	3) Growth and degradation rates higher where oxygen levels are higher	Bioaugmentation plot with oxygen shows higher activity and higher numbers of JS666	Degradation rates are higher. Cannot distinguish whether growth rates are higher
	4) Ease of use	Technology is easy to implement	Yes if only bioaugmentation and aeration; buffering is more time-consuming
Quantitative	1) Reduce cDCE concentrations	>75% reduction of cDCE concentrations in bioaugmented plots	No
	2) Greater reduction of cDCE in bioaugmented plots compared to control plot	Greater than 2x reduction of cDCE in bioaugmented plots compared to control plots	No
	3) Lower costs compared to pump and treat (P&T)	Average cost savings of 30-50% over P&T	Yes, if no buffer or aeration required

culture can be distributed, the fewer injection wells that are required for full-scale implementations.

JS666 was tracked in groundwater samples using two molecular probes (i.e., ISO and CMO). In addition, JS666 activity and presence were also evaluated through microcosm assays conducted using groundwater from the wells in each of the plots. Successful distribution is indicated by the presence and activity of JS666 in bioaugmented plots but not in control plots or background wells. JS666 spread downgradient and transgradient from the injection wells in the bioaugmented plots and was not identified in the upgradient or control wells to any significant degree. Therefore, the JS666 distribution objective was met.

3.3 IMPACT OF OXYGEN LEVELS ON GROWTH AND DEGRADATION RATES

For this performance objective, we originally planned to compare the impact of higher oxygen levels (relative to ambient) on the growth of JS666 and rate of cDCE degradation between the bioaugmented plots. The plan was to evaluate the effect of oxygen on growth by comparing JS666 levels using molecular probes and the degree of cDCE degradation by comparing changes in cDCE concentrations in bioaugmented plot wells, with and without oxygen addition. This performance objective would be successfully met if we had observed higher JS666 growth rates (i.e., higher CFUs/L over time) and higher rates of cDCE degradation in the oxygen-amended plot wells.

Despite the higher TCE concentrations in Bioaugmentation Plot #1, more biodegradation was observed in Bioaugmentation Plot #1 as illustrated by the higher degree of $\delta^{13}C$ enrichment measured using isotopic analyses (Figure 5-10). The higher degree of $\delta^{13}C$ enrichment may have been due to more biodegradation as a result of the added oxygen in IW-01.

Both Bioaugmentation Plot #1 and Plot #2 had relatively low levels of JS666 according to qPCR measurements. Therefore, the effect of oxygen on JS666 growth could not be evaluated.

3.4 EASE OF USE

The ease of use of the bioaugmentation culture, buffer and aeration equipment is an important factor in maintaining low operation costs for this technology. Ideally, the culture and amendment delivery can be conducted with minimal special training for operators and in a short period of time. The ease of use of this technology was evaluated based on our experience in the field with these bacteria and amendments.

Based on our experience with the field demonstration, bioaugmentation was easy, requiring no special measures, as was aeration and buffer amendment. Buffer injections were, however, time-consuming due to the lower permeability of this aquifer. Nevertheless, this performance objective was met and would definitely be met at sites with groundwater pH in the 6.5 to 8 range.

3.5 COST COMPARISON

The final quantitative objective was to compare the cost of a JS666 bioaugmentation remedy to a pump-and-treat system over a 30 year timeframe. A present value cost comparison between the two technologies was conducted, as discussed in Section 7.0. The criterion chosen for success was a present value cost-savings of 30-50% for the JS666 technology compared to pump and treat. The cost analysis showed a projected cost savings of 47%, assuming no aeration or buffering is required. Thus, under these assumptions, the JS666 technology is cost-effective when compared to pump and treat.

4.0 SITE DESCRIPTION

The field demonstration was conducted at Site 21, St. Juliens Creek Annex (SJCA) in Chesapeake, Virginia (the "Site"). This site has several relatively well-characterized groundwater plumes of chlorinated volatile organic compounds (VOCs; primarily cDCE, TCE and VC), appropriate site conditions, and a suitable on-site support network for execution of the demonstration. The rationale for the selection of this site is presented in the Site Selection Memorandum for Enhancing Natural Attenuation Through Bioaugmentation with Aerobic Bacteria that Degrade *cis*-1,2-DCE (Geosyntec, 2008).

In the following sections, the site location and history (Section 4.1), site geology/hydrogeology (Section 4.2), and contaminant distribution (Section 4.3) are discussed.

4.1 SITE LOCATION AND HISTORY

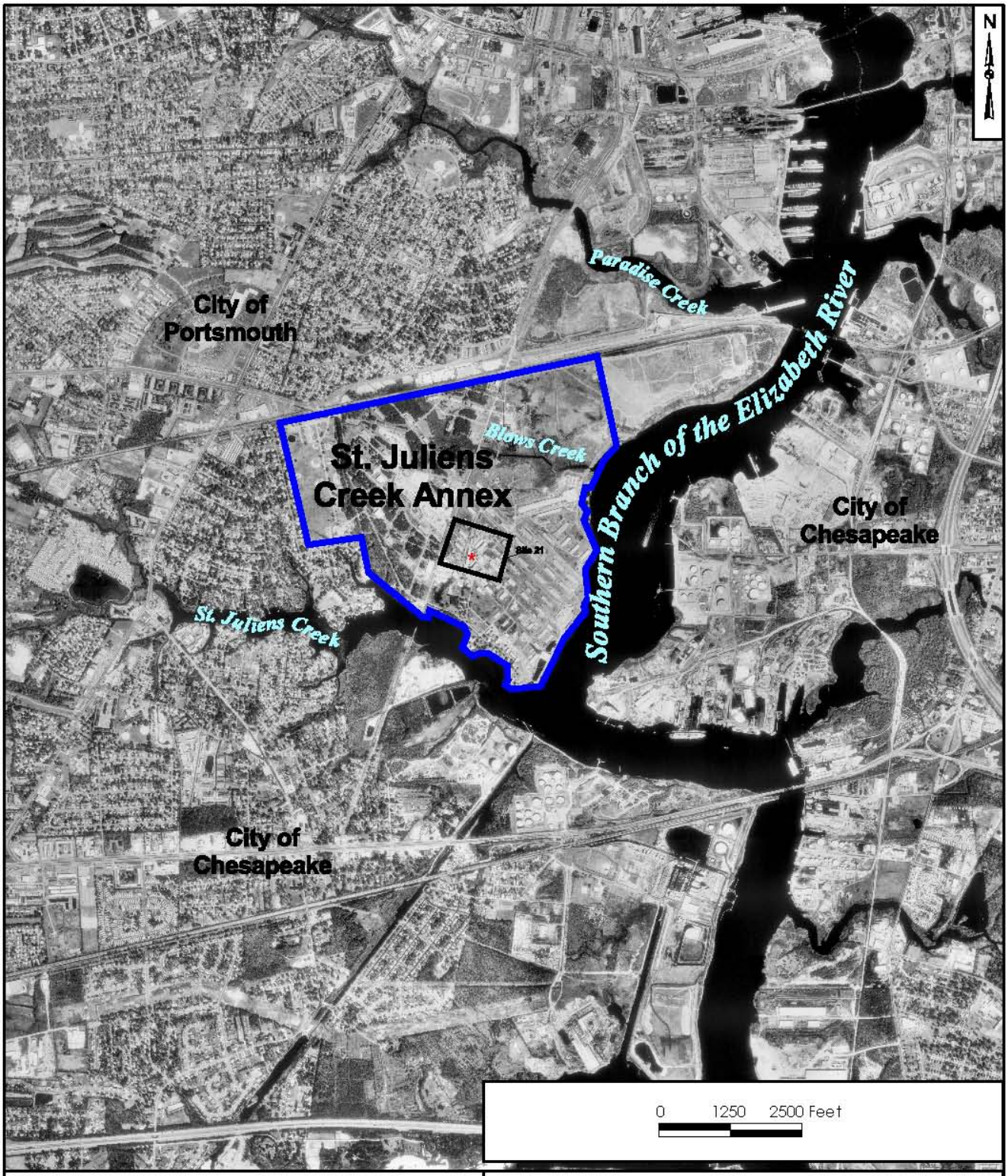
The site is located on St. Julien's Creek Annex (SJCA) Navy Depot, Site 21, in Chesapeake, Virginia. Information on the test site history, geology and hydrogeology is presented in the Remedial Investigation (RI) Report for Site 21 (CH2M HILL, 2008).

The SJCA facility covers approximately 490 acres and is located at the confluence of the St. Juliens Creek and the Southern Branch of the Elizabeth River in the City of Chesapeake. Most surrounding areas are developed and include residences, schools, recreational areas, and shipping facilities for several large industries. SJCA began operations in 1849 as a naval ammunitions facility, although ordnance operations ceased in 1977. SJCA currently acts as a radar-testing range and houses various administrative and warehousing facilities for the nearby Norfolk Naval Shipyard and other local naval activities (CH2M HILL, 2008). The Standard Industrial Classification (SIC) code for the current and former manufacturing activities at SJCA is 9711 (National Security).

The Site is located in a former industrial area in the south-central portion of SJCA (Figure 4-1). Buildings at the Site were historically used as machine, vehicle, and locomotive maintenance shops including paint shops, degreasing shops, electrical shops, and munitions loading facilities. However, many of the older buildings have been demolished. Outdoor areas were used for equipment and chemical storage. Solvents and other chemicals used at the Site were reportedly dumped on the ground outside the buildings for the purpose of dust and weed control. A former fuel service station was also located at the Site. Two abandoned underground storage tanks (USTs) with a history of leakage are located at the former fuel station (CH2M HILL, 2008). The location of the pilot test area (PTA) at the Site is shown in Figure 4-2.

4.2 SITE GEOLOGY/HYDROGEOLOGY

The Southeastern Virginia Coastal Plain physiographic province is underlain by an eastward thickening wedge of marine and non-marine sediments ranging in age from early Cretaceous to Holocene. Along the coastline, several thousands of feet of interlayered, unconsolidated



Legend

- St. Juliens Creek Annex
- Site 21
- ★ Demonstration Location

Reference:

CH2MHill. 2008. Final Remedial Investigation Report for Site 21: St. Juliens Creek Annex, Chesapeake, Virginia. CLEAN III Program Contract N62470-02-D-3052

location Map.ai

Location of Site 21 at St. Juliens Creek Annex
Site 21, St. Julien's Creek Annex, Chesapeake, VA

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Figure **4-1**

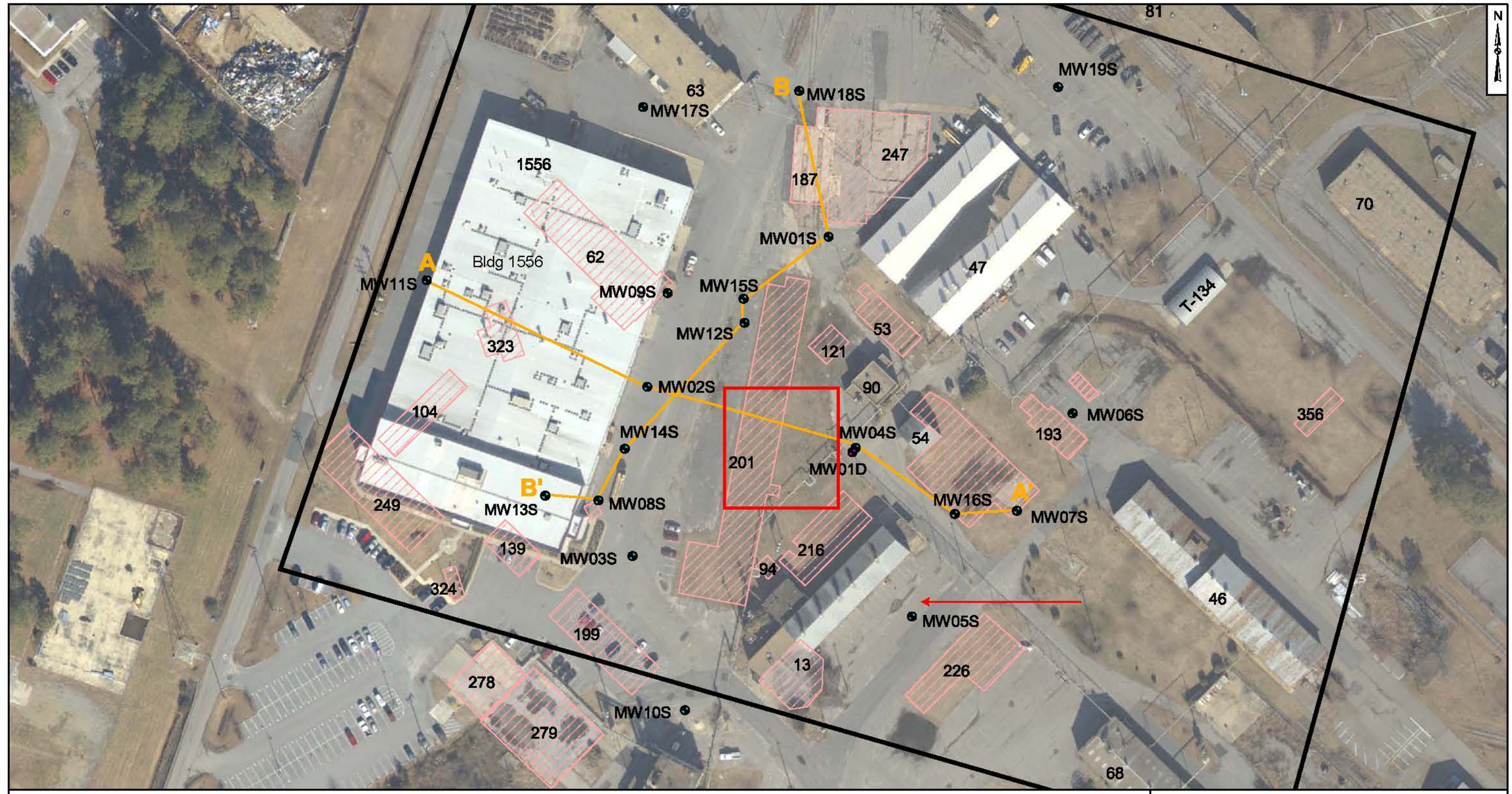
N
81
MW19S
63
MW17S
B
MW18S
247
187
1556
70
MW01S
A
Bldg 1556
62
47
MW11S
MW09S
MW15S
T-134
MW12S
53
121
323
MW02S
90
104
MW06S
356
MW14S
MW04S
54
193
201
MW01D
B'
MW16S
A'
249
MW13S
MW08S
MW07S
139
MW03S
216
94
324
46
MW05S
199
13
226
278
MW10S
279
68
Legend
Site 21 Boundary
Shallow Monitoring Well Location
Deep Monitoring Well Location
Cross Section Location
Demolished Buildings
Pilot Test Area
General Direction of Groundwater Flow
Reference:
CH2MHill. 2008. Final Remedial Investigation Report for Site 21: St. Juliens Creek Annex, Chesapeake, Virginia. LANTDIV CLEAN III Program Contract N62470-02-D-3052
0
100
200 Feet
Location of Pilot Test Area
Site 21, St. Julien's Creek Annex, Chesapeake, VA
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Figure 4-2

sediment, consisting of gravel, sand, silt, and clay deposits overlie pre-Cretaceous crystalline basement rock. Geologic units present beneath SJCA include (from youngest to oldest), Holocene deposits, undifferentiated Pleistocene deposits of the Columbia Group (Sand Bridge and Norfolk Formations), the Miocene to Pliocene Chesapeake Group of formations (including Yorktown Formation), the Paleocene to Eocene Pamunkey Group of formations (Nanjemoy Formation), late Cretaceous undifferentiated sediments, and the early to late Cretaceous Potomac Formation (CH2M HILL, 2008). For the purpose of this field demonstration only the Columbia Group and Chesapeake Group Formations are discussed.

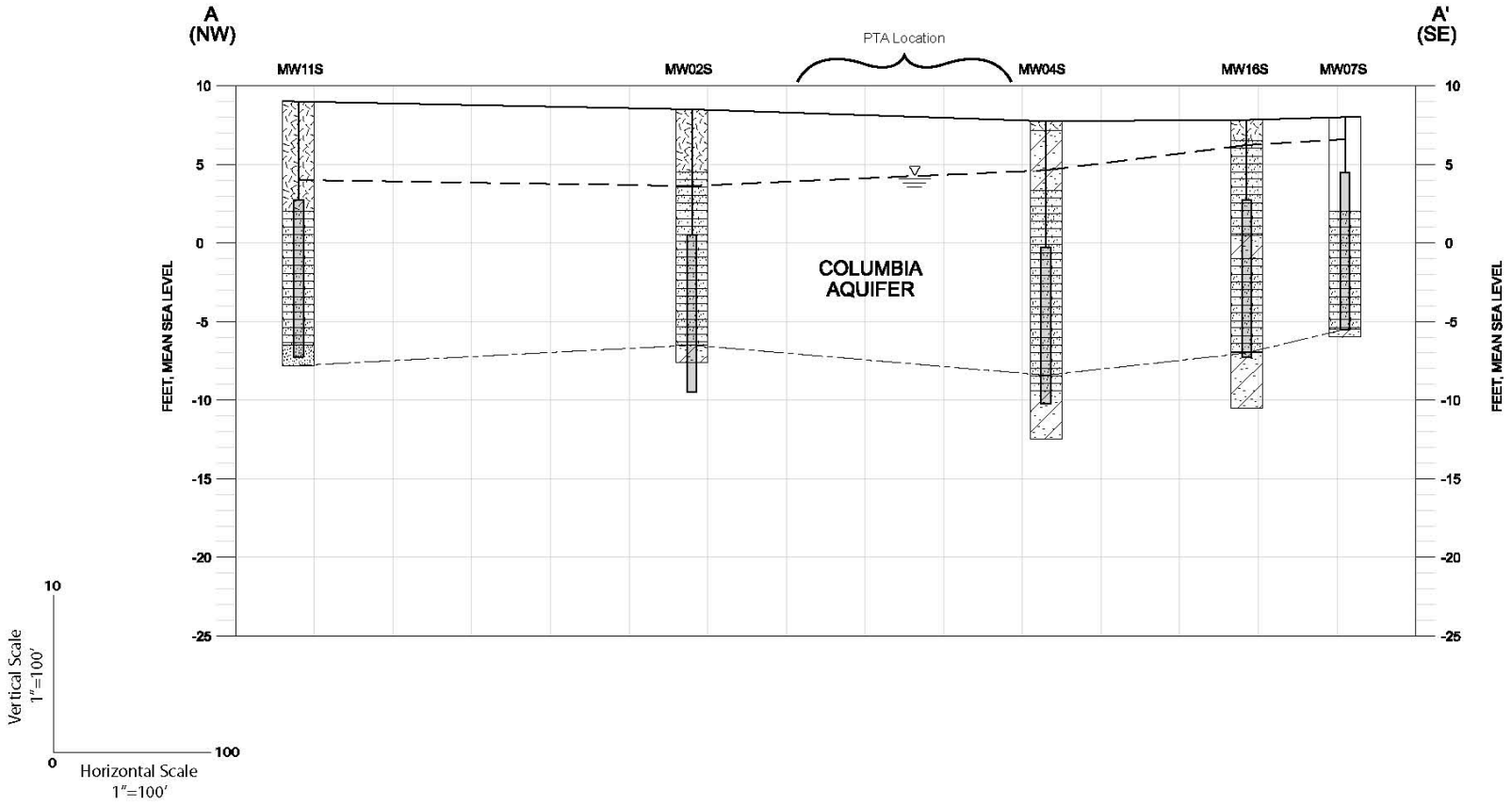
The Columbia Group, composed of Holocene deposits and undifferentiated Pleistocene deposits, is the uppermost geologic unit in the area and is approximately 60 feet (ft) thick. The upper 20 to 40 ft comprises the Columbia aquifer. Beneath the Site, the Columbia aquifer consists of brown and tan, fine to coarse, silty sand, ranging in thickness from approximately 13 to 20 ft. The lower 20 to 40 ft of the Columbia Group consists of relatively impermeable silt, clay, and sandy clay (CH2M HILL, 2008).

The Columbia Group is underlain by the Chesapeake Group. The uppermost unit in the Chesapeake Group is the Yorktown Formation and is approximately 300 to 400 ft thick with major sand, gravel, and shell beds in the upper 50 to 100 ft of the formation. Hydrostratigraphic units of the Yorktown Formation consist of the upper Yorktown confining unit and the lower Yorktown-Eastover aquifer (Yorktown aquifer). The Yorktown confining unit consists of blue-gray and green-gray fat clay. At the Site, the Yorktown confining unit was observed to have a thickness of 17 ft. The Yorktown aquifer underlies the Yorktown confining unit and consists of coarse gray sand with shelly hash (CH2M HILL, 2008). A generalized geologic cross section of the subsurface geology at the Site is presented in Figure 4-3 (the location of cross section A-A' at the Site is shown in Figure 4-2).

Groundwater at the site flows southwest in the eastern portions of the site and southeast in the western portions of the site toward the storm sewer system east of Building 1556. In the vicinity of the PTA, groundwater flow is towards the west. Shallow groundwater typically ranges from 2 to 7 ft below ground surface (bgs) (CH2M HILL, 2008). A potentiometric map for the Columbia aquifer is presented in Figure 4-4. Estimates of the hydraulic gradient and groundwater velocity for the Columbia aquifer are 0.004-0.01 ft/ft and 72 ft/yr, respectively (CH2M HILL, 2008).

4.3 CONTAMINANT DISTRIBUTION

Based on historical records and field investigation data, several source areas have been identified at the Site. These include an area west of demolished Building 201, an area west of demolished Building 187, an area between Building 47 and demolished Building 53, an area north of Building 47, an area northeast of Building 249, an area south of demolished Building 54, and the Building 46 Area (Figures 4-5 through 4-7) (CH2M HILL, 2008).



Legend

Monitoring Well Screened Interval

Water Table

Stratigraphy

Ground Surface

Hydrostratigraphic Contact

Interpolated Water Table

Lithology/USCS Description

Fill Material: Asphalt

Well Graded Sand

Fine to Medium Sand

Silty Clay

Reference:

CH2MHill. 2008. Final Remedial Investigation Report for Site 21: St. Juliens Creek Annex, Chesapeake, Virginia. CLEAN III Program Contract N62470-02-D-3052

Notes:

This cross section is interpertive and was prepared by interpolation between boring locations. Actual conditions between borings may differ from those shown here.

Generalized Geologic Cross Section

Site 21, St. Julien's Creek Annex, Chesapeake, VA

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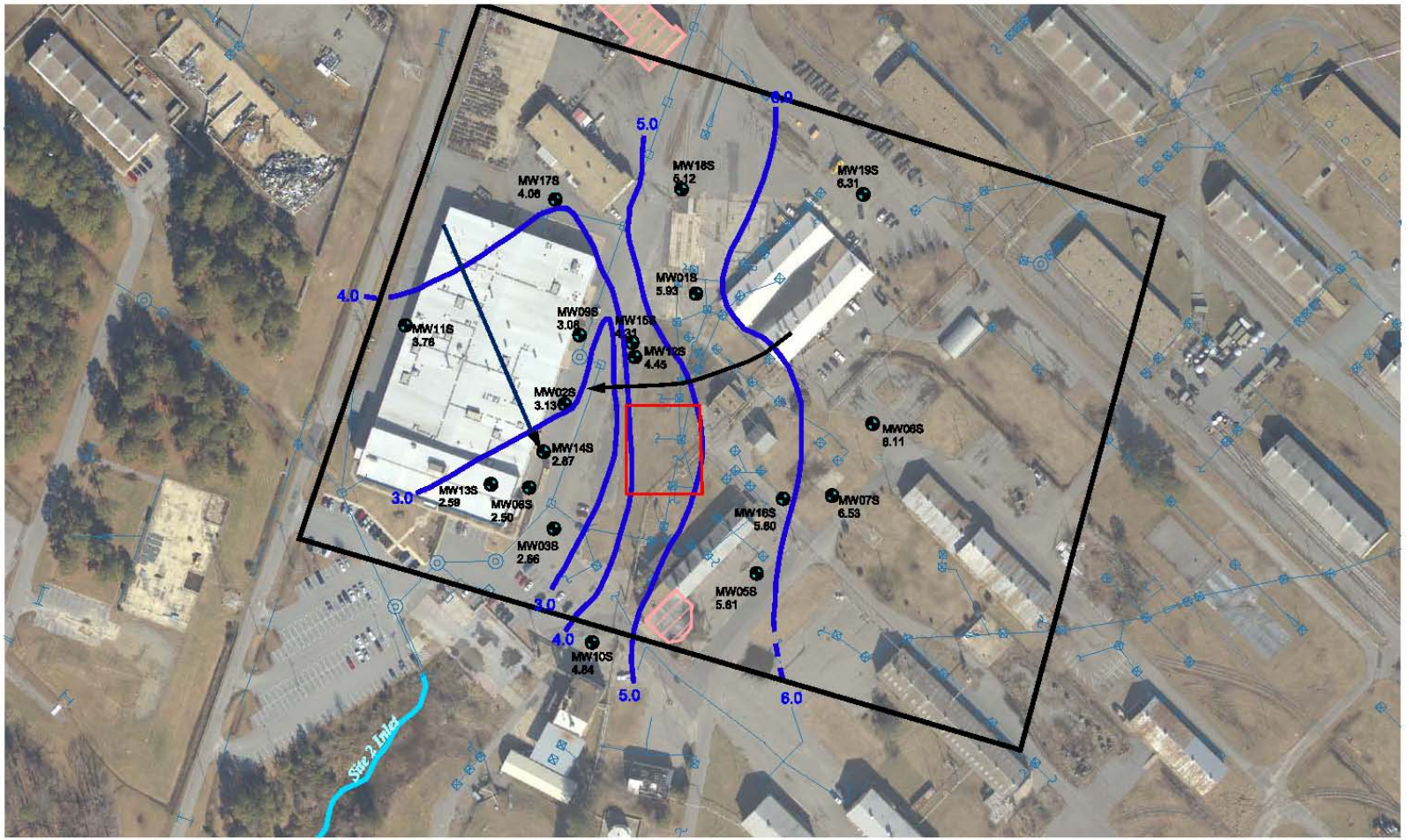
Figure **4-3**

Hydrostratigraphic Cross-Sections.cdr



Legend

- Site 21 Boundary
- Shallow Monitoring Well Location
- Estimated Groundwater Flow Direction
- Demolished Buildings
- Potentiometric Contour Lines (dashed when inferred)
- 3.28 Groundwater Elevation (feet above mean sea level)
- Storm Sewer System
- Pilot Test Area

Reference:

CH2MHill. 2008. Final Remedial Investigation Report for Site 21: St. Juliens Creek Annex, Chesapeake, Virginia. CLEAN III Program Contract N62470-02-D-3052

Notes:

Groundwater samples were collected: October and November 2005; October 2006 to January 2007; and February 2007.

0 100 200
Feet

Potentiometric Map of the Columbia Aquifer
Site 21, St. Julien's Creek Annex, Chesapeake, VA

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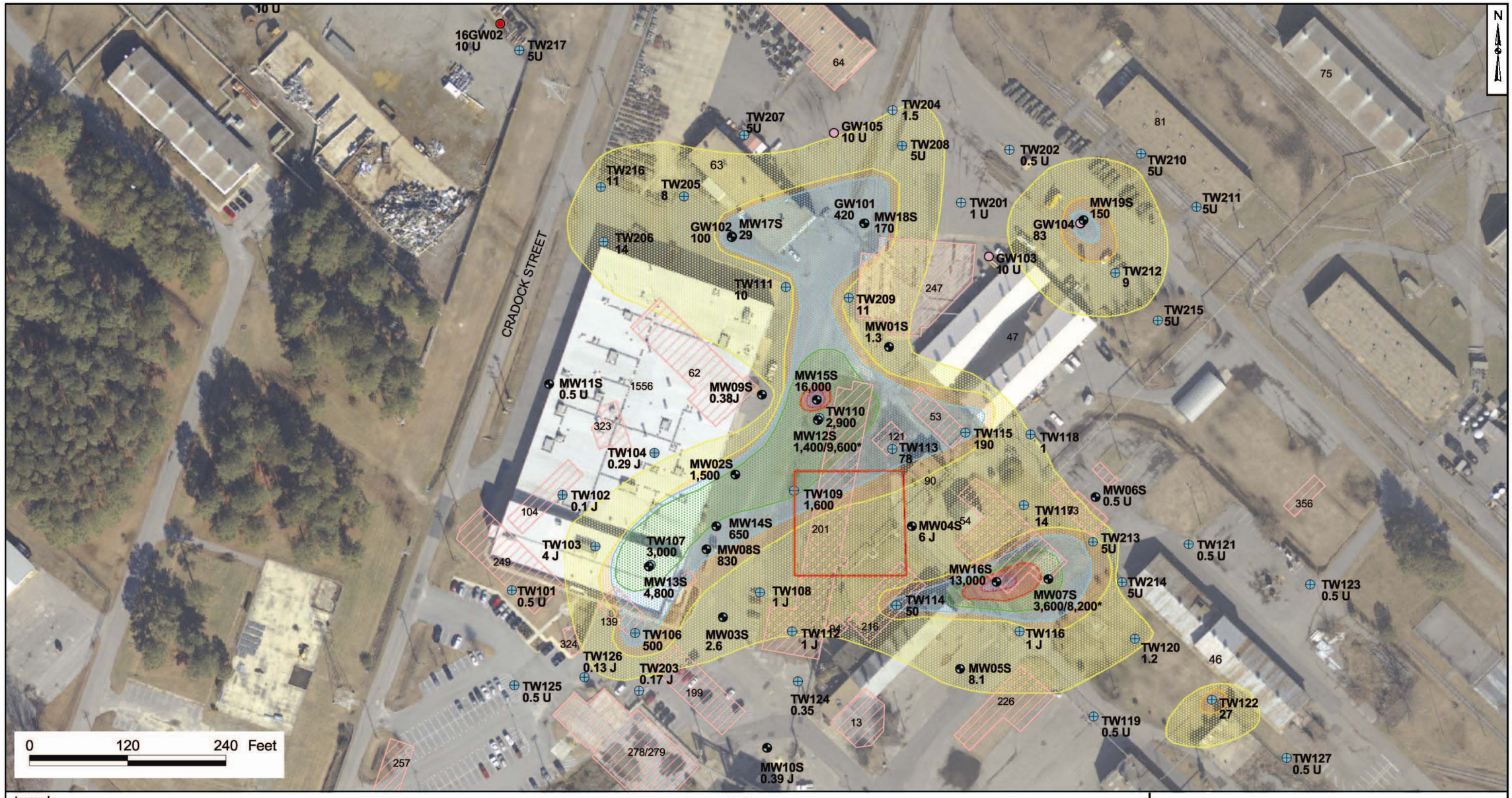
Figure **4-4**

surface map.ai



Legend

- Permanent Monitoring Well Location
- Temporary Monitoring Well Location
- Grab Groundwater Sample Location
- RRR Groundwater Sample Location
- Demolished Buildings
- Estimated Groundwater Flow Direction
- Proposed Pilot Test Area
- TCE Concentrations in µg/L

- J — Analyte detected, result is estimated
- U — No Detect
- Concentrations shown are the most recent results, with the exception of MW09S, at which a previous result was used because the most recent detection limit was above the MCL.
- * — Concentrations from depth specific DPT GW surplus collected at the bottom of the aquifer, not used to delineate the plume

- TCE Concentration 1 - 25
- TCE Concentration 26 - 49
- TCE Concentration 50 - 999
- TCE Concentration 1,000 - 4,999
- TCE Concentration 5,000 - 9,999
- TCE Concentration >= 10,000

Reference:

CH2MHill. 2008. Final Remedial Investigation Report for Site 21: St. Juliens Creek Annex, Chesapeake, Virginia. CLEAN III Program Contract N62470-02-D-3052

Notes:

Groundwater samples were collected: October and November 2005; October 2006 to January 2007; and February 2007.

Shallow Groundwater TCE Plume
Site 21, St. Julien's Creek Annex, Chesapeake, VA

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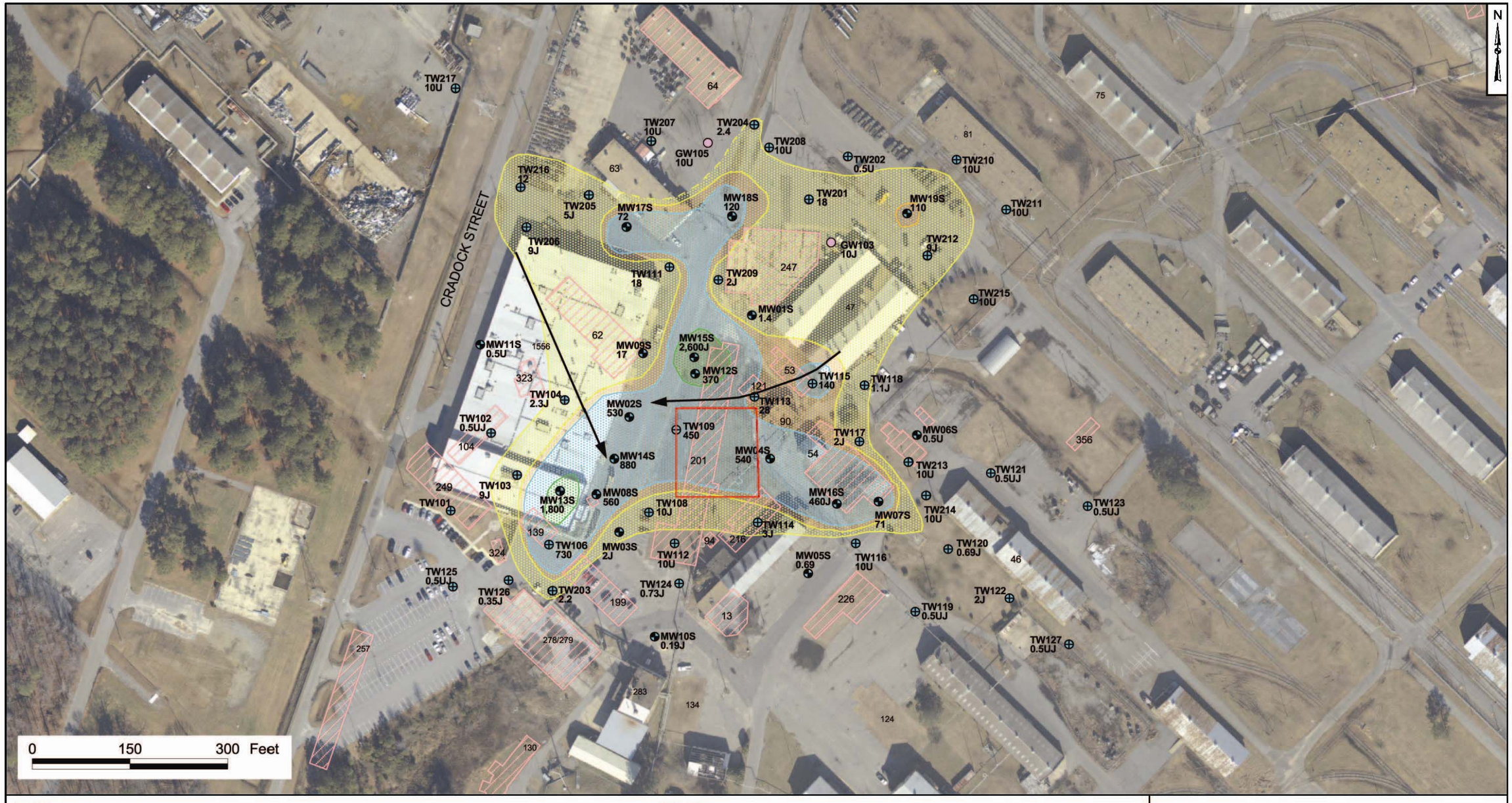
Figure **4-5**

Shallow Groundwater_TCE_2.ai



Legend

- Shallow Monitoring Well Location
- Temporary Monitoring Well Location
- Grab Groundwater Sample Location
- Demolished Buildings
- Estimated Groundwater Flow Direction
- Pilot Test Area

DCE Dichloroethene
U No Detect
J Reported value is estimated
All results are reported in µg/L
Concentrations shown are the most recent results.

- Cis-1,2-DCE Concentration 1 - 25
- Cis-1,2-DCE Concentration 26 - 49
- Cis-1,2-DCE Concentration 50 - 999
- Cis-1,2-DCE Concentration >=1,000

Reference:
CH2MHill. 2008. Final Remedial Investigation Report for Site 21: St. Juliens Creek Annex, Chesapeake, Virginia. LANTDIV CLEAN III Program Contract N62470-02-D-3052

Notes:
Groundwater samples were collected: October and November 2005; October 2006 to January 2007; and February 2007.

Shallow Groundwater cDCE Plume
Site 21, St. Julien's Creek Annex, Chesapeake, VA

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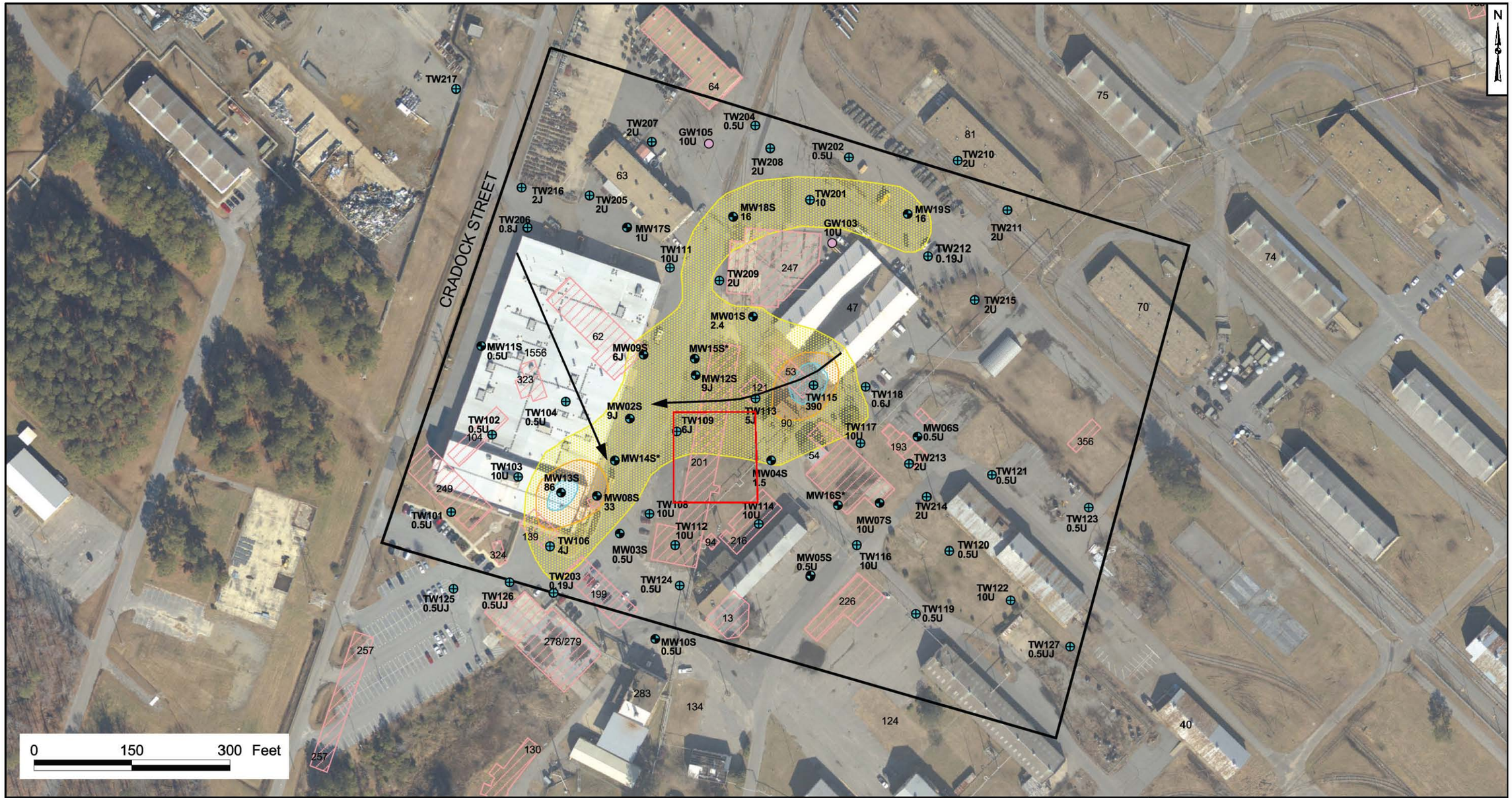
Figure 4-6



Legend

- Site 21 Boundary
- Shallow Monitoring Well Location
- Temporary Monitoring Well Location
- Grab Groundwater Sample Location
- Demolished Buildings
- Estimated Groundwater Flow Direction
- Pilot Test Area

- **VC** vinyl chloride
- **J** Reported value is estimated
- **U** No Detect

All results are reported in µg/L
Concentrations shown are the most recent results, with the exception of MW04S, at which a previous result was used because the most recent detection was above the MCL.

- ***** Results not used to delineate plume due to elevated detection limits from sample dilutions.

- VC Concentration 1 - 25
- VC Concentration 26 - 49
- VC Concentration > = 50

Reference:

CH2MHill. 2008. Final Remedial Investigation Report for Site 21: St. Juliens Creek Annex, Chesapeake, Virginia. CLEAN III Program Contract N62470-02-D-3052

Notes:

Groundwater samples were collected: October and November 2005; October 2006 to January 2007; and February 2007.

Shallow Groundwater VC Plume
Site 21, St. Julien's Creek Annex, Chesapeake, VA

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Figure **4-7**

Groundwater contamination appears to be confined to the surficial aquifer (Columbia aquifer) with some sorbed mass at the top of the Yorktown confining unit. The primary groundwater contaminants at the Site are TCE, cDCE, and VC. Isopleth maps for these contaminants from the most recent round of groundwater sampling conducted by CH2M Hill are shown in Figures 4-5 through 4-7. Upon review of this data, a potentially favorable demonstration area was identified around existing monitoring well MW04S where only cDCE was present at elevated concentrations and moderately aerobic conditions prevailed.

To confirm that appropriate groundwater conditions for a field demonstration were present in this area, a groundwater sample was collected from well MW04S in December 2007 and analyzed for VOCs and select geochemical parameters. Results of these analyses confirmed that suitable groundwater conditions exist. TCE, cDCE, and VC concentrations were <10 µg/L, 780 µg/L, and 2 µg/L, respectively (Table 4-1). Concentrations of other VOCs were either near or below analytical quantitation limits. The groundwater pH at well MW04S was observed to be 5.88 which, although being slightly lower than desired, could be adjusted through use of a buffering agent (phosphate buffer). The DO and the ORP levels were observed to be 1.65 mg/L and 79 mV, respectively (Table 4-2) and were indicative of moderately aerobic groundwater conditions.

TABLE 4-1: Pre-Demonstration VOC Analytical Results
Site 21, St. Julien's Creek Annex, Chesapeake, VA

Sample ID	SJS21-MW04S
Sample Date	11-Dec-07
Organic Compound (µg/L)	
dichlorodifluoromethane	10 U
chloromethane	10 U
vinyl chloride	**2 J**
bromomethane	10 U
chloroethane	10 U
trichlorofluoromethane	10 U
1,1-dichloroethene	10 U
1,1,2-trichloro-1,2,2-trifluoroethane	10 U
acetone	**13 B**
carbon disulfide	10 U
methyl acetate	10 U
methylene chloride	**2 JB**
trans-1,2-dichloroethene	**11**
methyl tert-butyl ether	10 U
1,1-dichloroethane	10 U
cis-1,2-dichloroethene	**780**
2-butanone	10 U
chloroform	10 U
1,1,1-trichloroethane	10 U
cyclohexane	10 U
carbon tetrachloride	10 U
benzene	10 U
1,2-dichloroethane	10 U
trichloroethene	10 U
methylcyclohexane	10 U
1,2-dichloropropane	10 U
bromodichloromethane	10 U
cis-1,3-dichloropropene	10 U
4-methyl-2-pentanone	10 U
toluene	10 U
trans-1,3-dichloropropene	10 U
1,1,2-trichloroethane	10 U
tetrachloroethene	10 U
2-hexanone	10 U
dibromochloromethane	10 U
1,2-dibromoethane	10 U
chlorobenzene	10 U
ethylbenzene	10 U
xylene (total)	10 U
styrene	10 U
bromoform	10 U
isopropylbenzene	10 U
1,1,2,2-tetrachloroethane	10 U
1,3-dichlorobenzene	10 U
1,4-dichlorobenzene	10 U
1,2-dichlorobenzene	10 U
1,2-dibromo-3-chloropropane	10 U
1,2,4-trichlorobenzene	10 U

Notes:
Bold - detected compound
µg/L - micrograms per liter
U - compound analyzed but not detected at a concentration above the reporting limit
J - estimated value
B - analyte found in the sample and associated method blank

TABLE 4-2: Pre-Demonstration Water Quality Parameters
Site 21, St. Julien's Creek Annex, Chesapeake, VA

Sample ID	SJS21-MW04S
Sample Date	11-Dec-07
Parameter	
Dissolved Oxygen (mg/L)	1.65
Oxidation Reduction Potential (mV)	79
pH	5.88
Specific Conductance (mS/cm)	0.199
Temperature (°C)	20.69
Turbidity (NTU)	90
Salinity	0.01
Sulfate (mg/L)	26.9
Dissolved Iron (µg/L)	3880

Notes:
mg/L - milligrams per liter
µg/L - micrograms per liter
mV - millivolts
mS/cm - milliSiemens per centimeter
°C - degrees Celsius
NTU - nephelometric turbidity units

5.0 TEST DESIGN

The following sections provide a description of the conceptual experimental design, site-specific treatability studies, the design and layout of the technology components, field activities, groundwater sampling methods, analytical methods, and test results.

5.1 CONCEPTUAL EXPERIMENTAL DESIGN

For this demonstration, the site was instrumented to create four test plots within the pilot test area: a bioaugmentation plot receiving JS666, oxygen and buffer (Plot #1); a bioaugmentation plot receiving JS666 and buffer (Plot #2); a control plot receiving buffer (Plot #3); and a control plot receiving oxygen and buffer (Plot #4) as shown in Figures 5-1 and 5-2. The intent of the two bioaugmentation plots was to establish the effect of adding JS666 and additional oxygen on the rate of biodegradation, while the corresponding control plots were intended to account for the effects of buffer and buffer and oxygen on the results in the bioaugmentation plots. Two upgradient wells (MW-11 and MW-7) served as background controls to monitor the groundwater in the absence of amendments.

5.2 TREATABILITY STUDIES

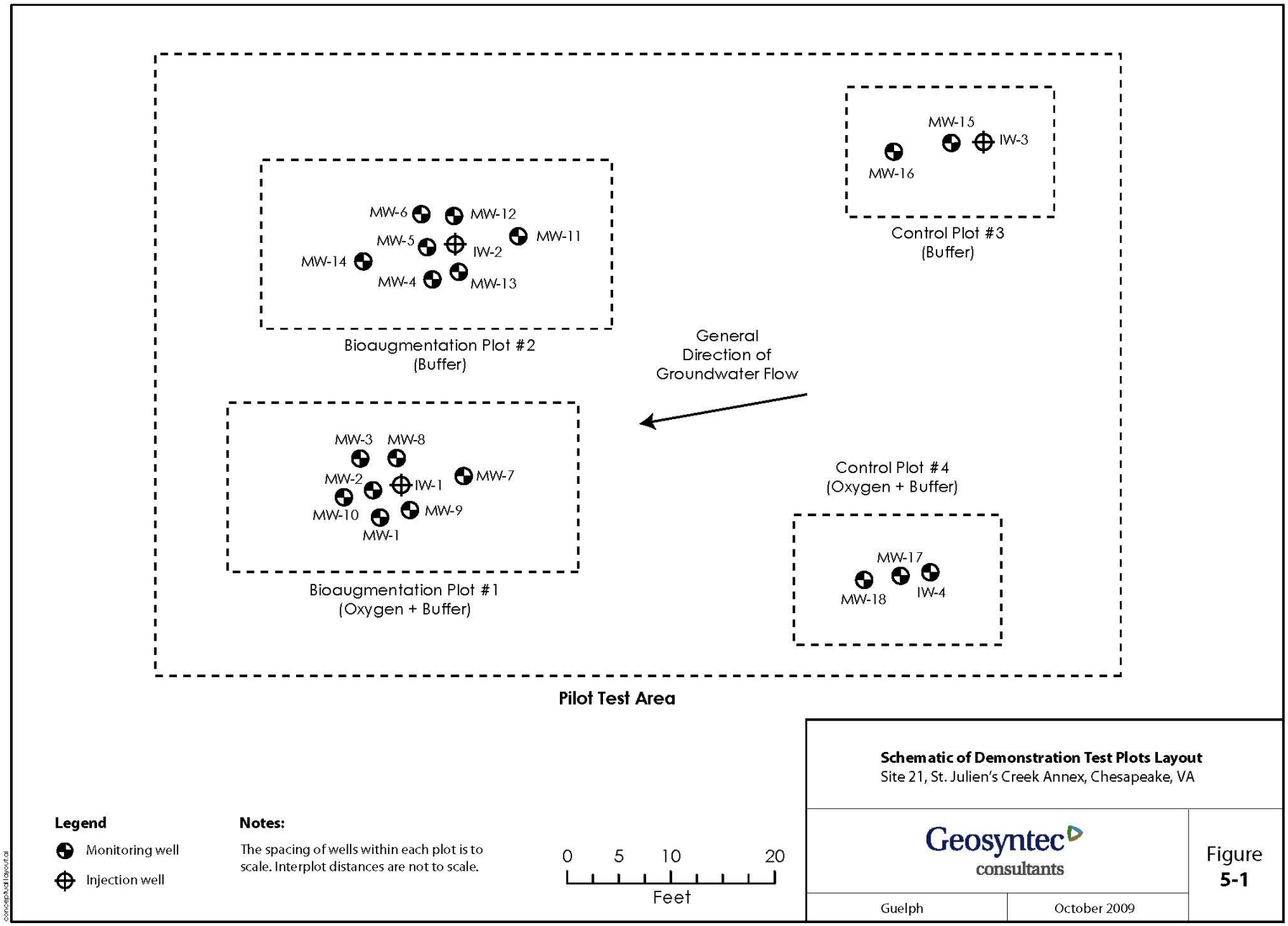
Treatability tests included site-specific microcosm studies and titration experiments as described in the following subsections. These studies were conducted at Cornell University.

5.2.1 Microcosm Studies with Site Groundwater

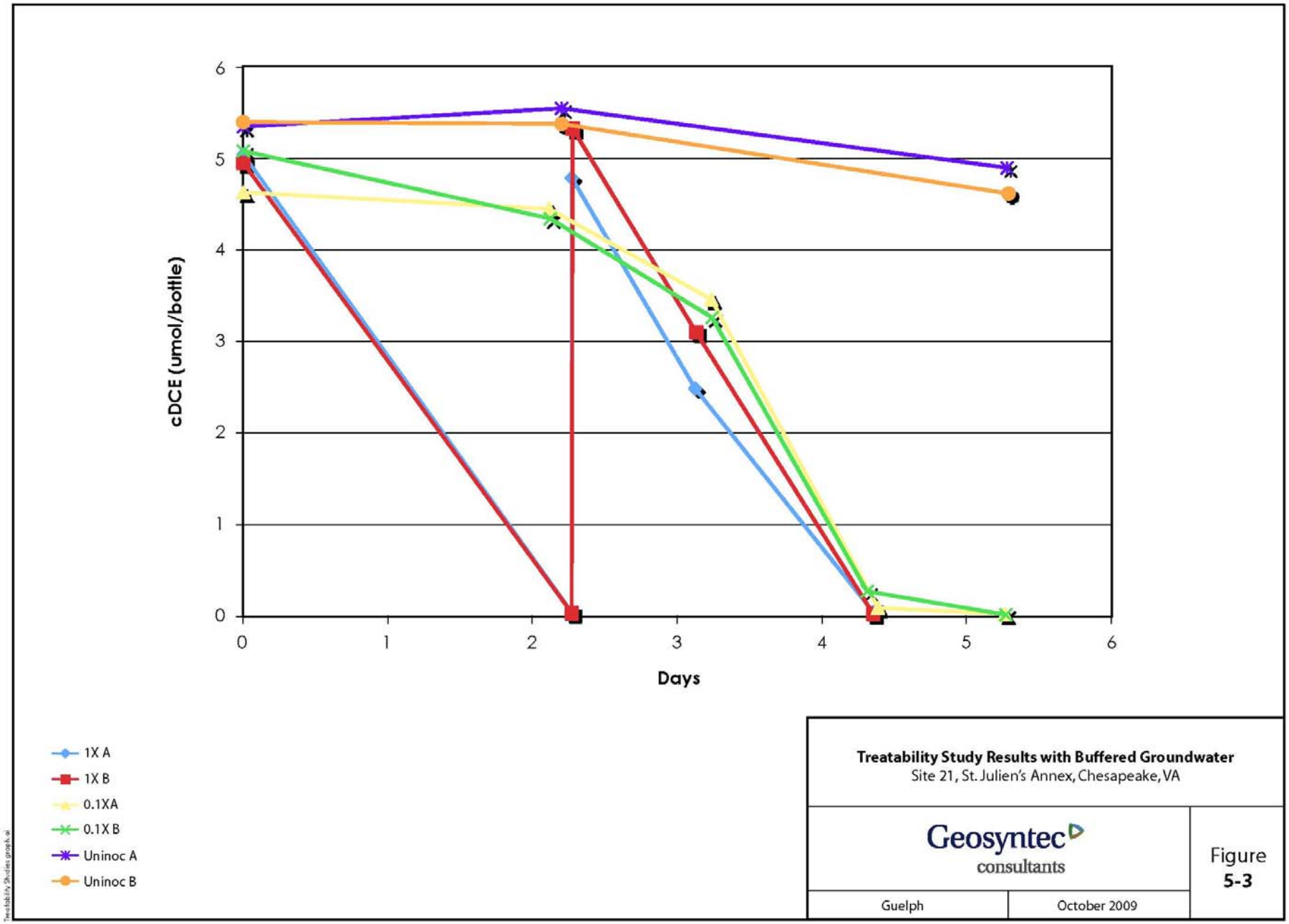
Microcosms using site groundwater were prepared in 160-mL serum bottles. Because of the low initial pH of the site groundwater (pH of 5.65), the groundwater was buffered to a pH of 7 using a phosphate buffer. Each microcosm was set up in duplicate and then dosed with 0.15 mL of a 25 mM cDCE solution for a final nominal concentration of approximately 11 mg/L (5.9 umol/bottle).

JS666 inoculum was obtained from active transfer cultures exhibiting growth on cDCE. Microcosms were inoculated with JS666 to achieve roughly either 3.5×10^8 ("1X") or 3.5×10^7 ("0.1X") organisms per bottle. An uninoculated control was also run in duplicate for comparison.

All 1X- and 0.1X-inoculated microcosms with buffered groundwater degraded all the cDCE present within 2 and 4 days, respectively, as shown in Figure 5-3. There was no degradation in any of the uninoculated controls.


MW-15
IW-3
MW-16
Control Plot #3
(Buffer)
MW-6
MW-12
MW-5
IW-2
MW-11
MW-14
MW-4
MW-13
Bioaugmentation Plot #2
(Buffer)
General
Direction of
Groundwater Flow
MW-3
MW-8
MW-2
IW-1
MW-7
MW-10
MW-9
MW-1
Bioaugmentation Plot #1
(Oxygen + Buffer)
Control Plot #4
(Oxygen + Buffer)
MW-17
MW-18
IW-4
Pilot Test Area
Legend
Monitoring well
Injection well
Notes:
The spacing of wells within each plot is to scale. Interplot distances are not to scale.
0
5
10
20
Feet
Schematic of Demonstration Test Plots Layout
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Guelph
October 2009
Figure
5-1
conceptual layout.ai


MW-16
MW-15
IW-03
N
MW-06
MW-12
MW-05
IW-02
MW-11
MW-14
MW-04
MW-13
MW-03
MW-08
MW-07
MW-02
IW-01
MW-10
MW-09
MW-01
MW-18
MW-17
IW-04
20
10
0
20 Feet
Legend
Monitoring Well
Injection Well
Notes
Aerial Photo copyright Microsoft Corporation 2009
PTA Well Locations
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Guelph
13-Oct-2009
Figure
5-2


cDCE (umol/bottle)
Days
0
1
2
3
4
5
6
1X A
1X B
0.1XA
0.1X B
Uninoc A
Uninoc B
Treatability Study Results with Buffered Groundwater
Site 21, St. Julien's Annex, Chesapeake, VA
Geosyntec consultants
Guelph
October 2009
Figure 5-3

5.2.2 Titration Studies

To assist in determining an appropriate buffer for the JS666 field study, the equivalents of strong base (NaOH) required to titrate site groundwater to pH 7.1 was experimentally determined to be 1.25 milliequivalents per liter (meq/L).

Next, the alkalinity of site groundwater was estimated by titrating to the CO_2-equivalence point with 0.02N H_2SO_4. The endpoint was visually determined to be pH 4.4 by examination of the shape of the titration curve, yielding an alkalinity of about 0.9 meq/L. The shape of the curve suggested that there were no significant acidic/basic species other than bicarbonate.

In the field, however, it is not advisable to neutralize with a strong base as invariably the pH would be overshot in such a weakly buffered system. Therefore, the amount of phosphate buffer (equimolar mixture of mono-basic and dibasic forms of orthophosphate) required to titrate site groundwater to pH 7.0 was determined. The result was 10 mM of the phosphate buffer. 20 to 30 mM of phosphate buffer was recommended for the bioaugmentation, as these levels were consistent with what the culture could tolerate and would provide some excess buffer to adjust the native groundwater pH.

5.3 DESIGN AND LAYOUT OF TECHNOLOGY COMPONENTS.

5.3.1 Construction and Installation of Wells

The monitoring network for each of the bioaugmentation plots consisted of one fully screened injection well and 7 fully screened monitoring wells (one well upgradient of the injection well, 2 wells transgradient to the injection well, and 4 wells downgradient of the injection well). The control plots were comprised of a smaller well network of one fully screened injection well and 2 fully screened downgradient monitoring wells, located upgradient and transgradient to the bioaugmentation plots (Figures 5-1 and 5-2).

For this demonstration it was envisioned that the wells would be spaced so as to accommodate a groundwater travel time of 2-6 weeks between adjacent wells (2-4 weeks between adjacent injection and downgradient/transgradient monitoring wells in all plots, and 4-6 weeks between the upgradient wells and injection wells in the bioaugmentation plots).

In an attempt to confirm groundwater direction and flow velocities before all wells were installed, well installations were performed in two separate mobilizations. During the first mobilization, the four injection wells and the first row of downgradient monitoring wells in the bioaugmentation plots were installed and a conservative tracer study was performed as discussed in Section 5.3.2. The remaining demonstration wells were installed during the second mobilization, following the tracer study.

Under the direction of a Geosyntec project geologist, boreholes were drilled to a maximum depth of 20 ft bgs by a licensed driller using hollow stem augers (HSA). During drilling,

continuous soil sampling was performed at select locations for the purpose of lithologic characterization.

Each fully screened monitoring well was constructed in a similar manner to the nearby existing monitoring well MW04S, using 2-inch diameter Schedule 40 polyvinyl chloride (PVC) screen (#10 slot) and Schedule 40 PVC riser. The four injection wells were constructed using 4-inch diameter Schedule 40 PVC screen (#10 slot) and Schedule 40 PVC riser. The screened interval of all wells was from approximately 8 to 18 ft bgs. After placement of the well screen and riser casing, the annular space around the casing was filled with uniformly graded, rounded, clean #1 silica sand to a depth of approximately 2 ft above the well screen. The height to the top of the filter pack was frequently measured to check that the volume of sand placed in the wells approximated the volume required for the annulus and that no bridging of the filter pack had occurred. The annular space above the sand pack was filled with bentonite pellets to a depth of approximately 1 ft bgs. All 2-inch monitoring wells, along with injection wells IW-02 and IW-03, were completed at surface with a steel, flush-mount, 8-inch protective casing set in a 2 ft x 2 ft concrete pad. The concrete pad was constructed using Portland Cement mixed with water according to the manufacturer's specifications. The surface of the concrete pads was graded to drain water away from the well. The riser casings were capped with a watertight end cap (J-plug). Surface completions for wells IW-01 and IW-04 consisted of a 2 ft x 2 ft x2 ft steel, flush-mount protective vault to house the air tanks for the oxygen emitters (see Section 5.3.3). Well construction details are summarized in Table 5-1.

Following installation, the new wells were developed by standard surging and purging methods. Borehole logs and well construction diagrams and survey information can be found in Appendix B.

Soil cores collected during well installations revealed the following soil lithology beneath each plot:

- Plot #1 consists primarily of grayish/brown to tan, fine to medium grained sand and silty sand to a maximum cored depth of 20ft bgs. A silty clay layer was found in cores collected from wells IW-1 and MW-2 from approximately 15 to 18 ft bgs, which is within the screened interval of these wells;

- Plot #2 consists primarily of grayish/brown to tan, fine to medium grained sand with some interbedded layers of silty sand to a maximum cored depth of 20ft bgs. A silty clay layer was found in cores collected from wells MW-13 and MW-14 from approximately 16 to 20 ft bgs, which is within and just below the screened interval of these wells;

- Plot #3 consists primarily of light grey to tan fine to medium grained sand to a maximum cored depth of 20ft bgs; and

- Plot #4 consists primarily of light grey to brown/orange fine to medium grained sand with some interbedded layers of silty sand to a maximum cored depth of 18ft bgs.

TABLE 5-1: WELL CONSTRUCTION DETAILS
Site 21, St. Julien's Creek Annex, Chesapeake, VA

Well ID	Installation Date	Drilling Method*	Total Depth (ft bgs)	Top of Screen (ft bgs)	Bottom of Screen (ft bgs)	Borehole Diameter (inches)	PVC Casing Diameter (inches)	Ground Surface (ft amsl)	Top PVC Casing (ft amsl)	Coordinates (Northings)	Coordinates (Eastings)
IW-01	17-Sep-08	HSA	18	8.0	18.0	10	4	8.55	7.85	3453278.58	12123062.78
IW-02	18-Sep-08	HSA	18	8.0	18.0	10	4	8.63	8.38	3453330.08	12123068.01
IW-03	18-Sep-08	HSA	18	8.0	18.0	10	4	8.10	7.64	3453342.17	12123134.93
IW-04	18-Sep-08	HSA	18	8.0	18.0	10	4	7.82	7.48	3453246.08	12123129.70
MW-01	16-Sep-08	HSA	18	8.0	18.0	8.0	2	8.59	8.08	3453275.41	12123060.76
MW-02	16-Sep-08	HSA	18	8.0	18.0	8.0	2	8.64	8.13	3453278.03	12123060.09
MW-03	16-Sep-08	HSA	18	8.0	18.0	8.0	2	8.59	8.19	3453281.12	12123058.84
MW-04	17-Sep-08	HSA	18	8.0	18.0	8.0	2	8.66	8.09	3453326.73	12123065.77
MW-05	17-Sep-08	HSA	18	8.0	18.0	8.0	2	8.61	8.35	3453329.75	12123065.28
MW-06	17-Sep-08	HSA	18	8.0	18.0	8.0	2	8.73	8.44	3453332.86	12123064.71
MW-07	23-Oct-08	HSA	18	8.0	18.0	8.0	2	8.25	8.03	3453279.38	12123068.84
MW-08	23-Oct-08	HSA	18	8.0	18.0	8.0	2	8.35	8.12	3453281.11	12123062.33
MW-09	23-Oct-08	HSA	18	8.0	18.0	8.0	2	8.51	8.28	3453276.14	12123063.57
MW-10	23-Oct-08	HSA	18	8.0	18.0	8.0	2	8.63	8.33	3453277.32	12123057.31
MW-11	22-Oct-08	HSA	18	8.0	18.0	8.0	2	8.63	8.40	3453330.79	12123074.02
MW-12	22-Oct-08	HSA	18	8.0	18.0	8.0	2	8.62	8.34	3453332.66	12123067.82
MW-13	22-Oct-08	HSA	18	8.0	18.0	8.0	2	8.63	8.35	3453327.44	12123068.36
MW-14	22-Oct-08	HSA	18	8.0	18.0	8.0	2	8.68	8.31	3453328.43	12123059.13
MW-15	21-Oct-08	HSA	18	8.0	18.0	8.0	2	8.10	7.85	3453342.08	12123131.78
MW-16	21-Oct-08	HSA	18	8.0	18.0	8.0	2	8.14	7.79	3453341.28	12123126.18
MW-17	21-Oct-08	HSA	18	8.0	18.0	8.0	2	7.69	7.39	3453245.75	12123126.87
MW-18	21-Oct-08	HSA	18	8.0	18.0	8.0	2	7.64	7.28	3453245.33	12123123.34

Notes:
HSA - Hollow Stem Augers
* - Drilling conducted by Parratt Wolff Inc.
ft - feet
amsl - above mean sea level
bgs - below ground surface

5.3.2 Tracer Tests

Following the installation of wells during the first mobilization, a conservative tracer study was conducted in both bioaugmentation plots to confirm the direction of groundwater flow and assess the travel time between wells and the groundwater seepage velocity. An iodide tracer was used in proposed Bioaugmentation Plot#1, and a bromide tracer was used in proposed Bioaugmentation Plot #2. Each tracer was added to its respective test plot (via the injection well) at a sufficient concentration above background to generate breakthrough profiles at the downgradient monitoring wells. The following subsections outline the tracer test procedure and sampling methods and the results.

Tracer Test Procedures and Sampling Methods

Forty-five (45) gallons (gal) of groundwater were extracted from each of the injection wells in the bioaugmentation plots (IW-01 and IW-02) and stored in 55-gal polypropylene drums. Potassium iodide salt (223 grams) was dissolved in extracted groundwater from IW-01, and sodium bromide salt (219 grams) was dissolved in extracted groundwater from IW-02. Once fully dissolved, the tracer solutions were mixed into their respective 55-gal drums to achieve an approximate concentration of 1,000 mg/L. The concentrations in the drum were measured using a Thermo Orion 290A+ with either a Cole Parmer ISE Bromide Probe or Cole Parmer ISE Iodide Probe, and samples were collected for laboratory analysis. Calibration procedures for the two probes are discussed in Appendix D.

The tracer solutions were pumped back into their respective injection wells at a rate of approximately 0.7 liters per minute (L/min) over a 4 hour period to minimize mounding and prevent large increases in the natural gradient. Once all of the tracer solution was added, groundwater samples were collected from the injections wells for laboratory analysis and for field measurements using the probes.

An ISCO 6712 Full-Size Portable Sampler (Autosampler) was set up at the two monitoring wells (MW-02 and MW-05) directly downgradient from injection wells IW-01 and IW-02, respectively, and the sampling tubing was positioned at the mid-point of the screen (13 ft bgs) in monitoring wells MW-02 and MW-05. The autosamplers were calibrated (Appendix D) and programmed to collect a sample every 6 hours until the tracer was detected. Thereafter, the autosamplers collected a sample every 4 hours. Samples were collected from the four transgradient wells (MW-01, MW-03, MW-04, and MW-06) twice a day for the duration of the tracer test. The bromide and iodide concentrations were periodically measured using the bromide and iodide probes. Sampling was conducted until concentrations plateaued and began to decline (approximately 10-14 days). Ten (10) to eleven (11) samples per plot were submitted to the laboratory for bromide and iodide analysis to verify probe measurements. The bottles were decontaminated following the procedures outlined in Appendix D.

Results

The iodide and bromide concentrations in the drums prior to injection were measured to be 921.3 and 880.7 mg/L, respectively, using the probes. The concentrations of iodide and bromide in injection wells IW-01 and IW-02 after injection were 936 and 1,050 mg/L, respectively, as analyzed by the laboratory. Figure 5-4 and 5-5 illustrate the emerging iodide and bromide concentrations in the downgradient monitoring wells. During the tracer test, a significant rainfall event between September 25th and 26th resulted in surface water pouring into MW-05, causing the bromide concentrations in MW-05 to fall below detectable levels; however, the bromide concentrations began to rebound within 24 hours of the rain event (Figure 5-5). A table summarizing the analytical tracer data is located in Appendix E.

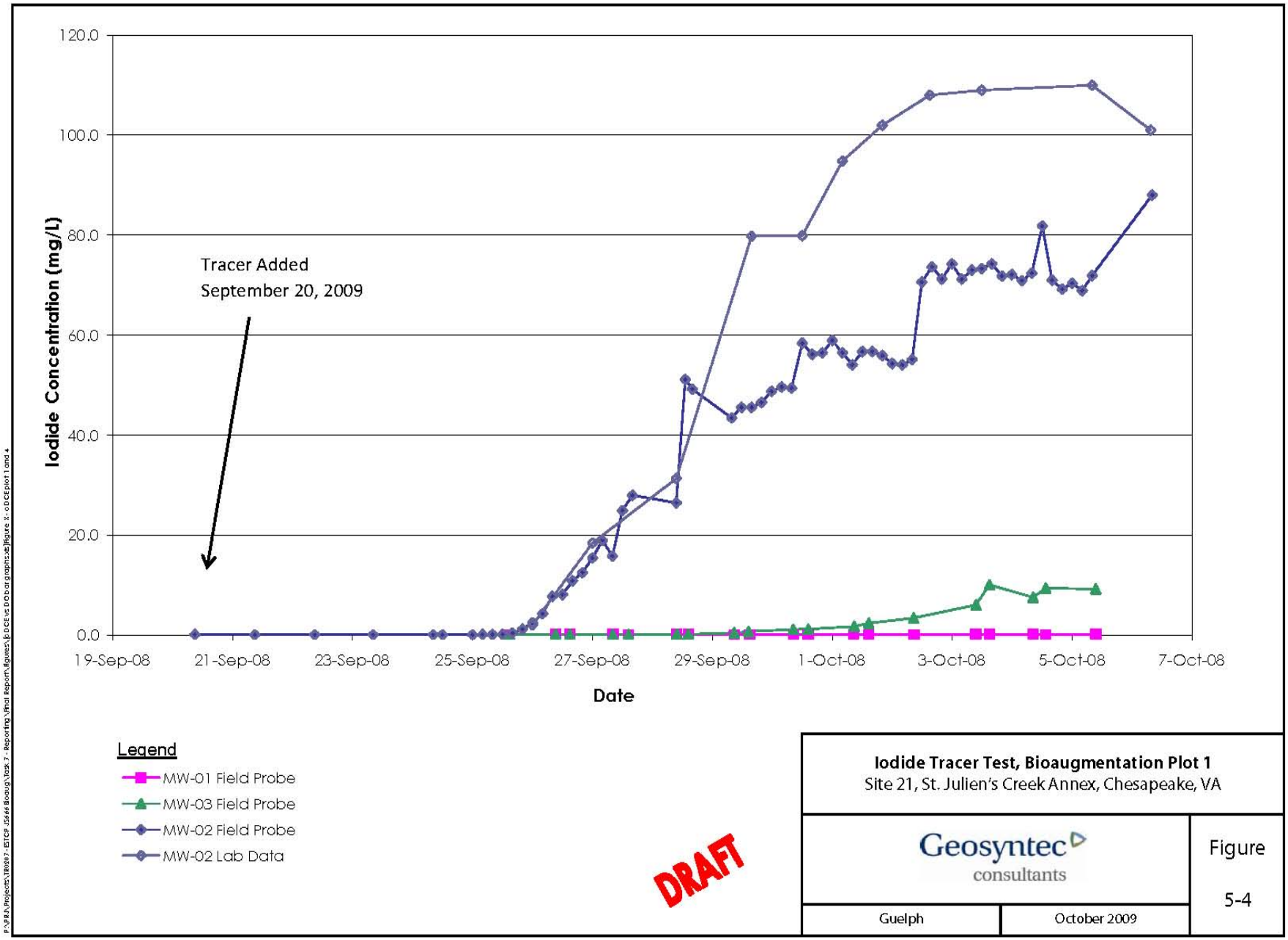
The results of the tracer tests confirmed the monitoring wells in the bioaugmentation plots were positioned downgradient from the injection wells. In Plot #1, the iodide tracer test was terminated just as concentrations began to decline in MW-02. As such, the residence time between IW-01 and MW-02 was estimated to be between 13 and 14 days. The bromide tracer test concentrations in Plot 2 plateaued and began to decline indicating a travel time between IW-02 and MW-05 of approximately 12 days. The groundwater flow rate was estimated from the results of the tracer test to be between 72 and 84 feet per year (ft/year), which is similar to the rate of 72 ft/year estimated by CH2M Hill (Section 4.2).

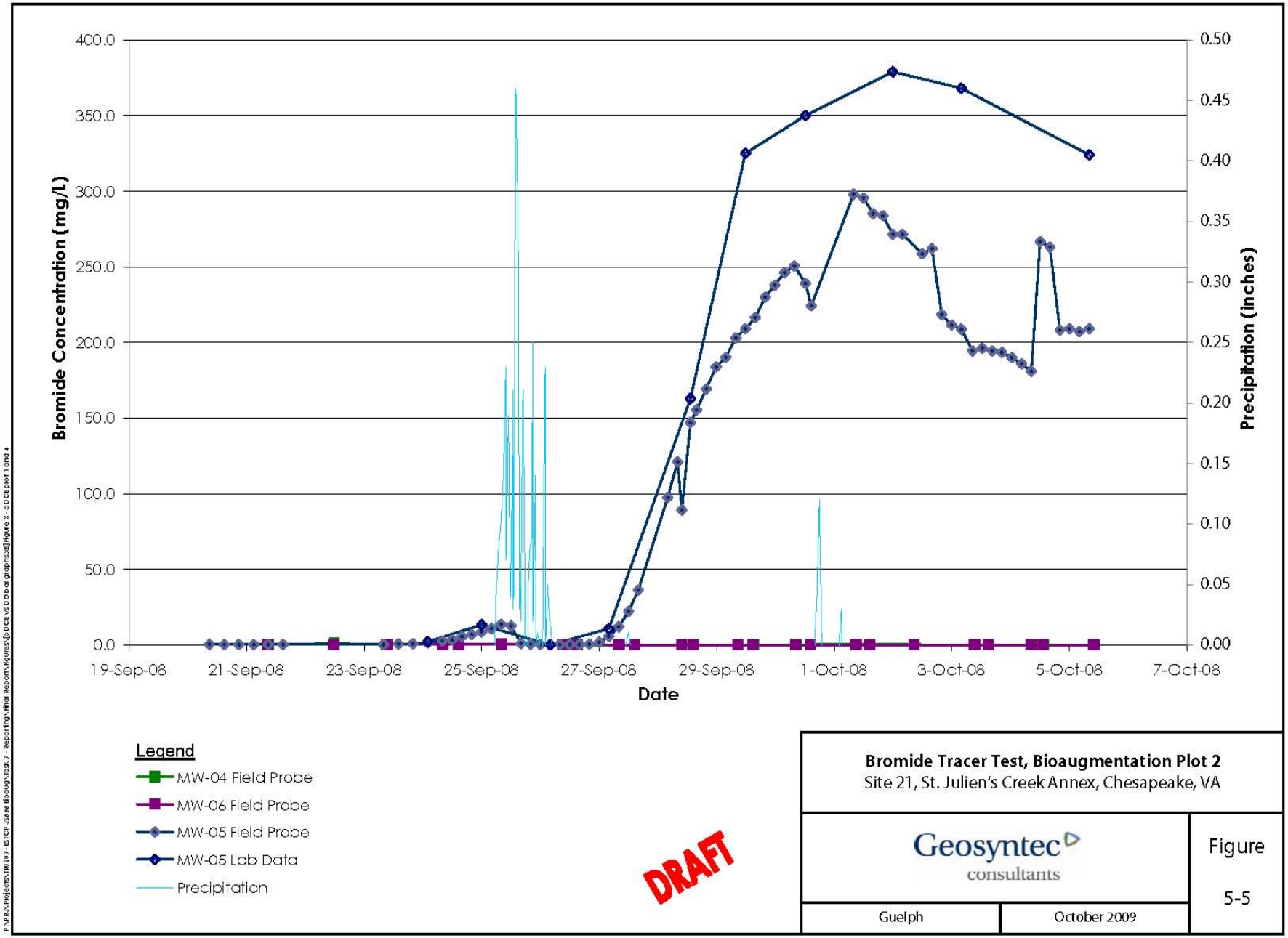
5.3.3 Aeration Device

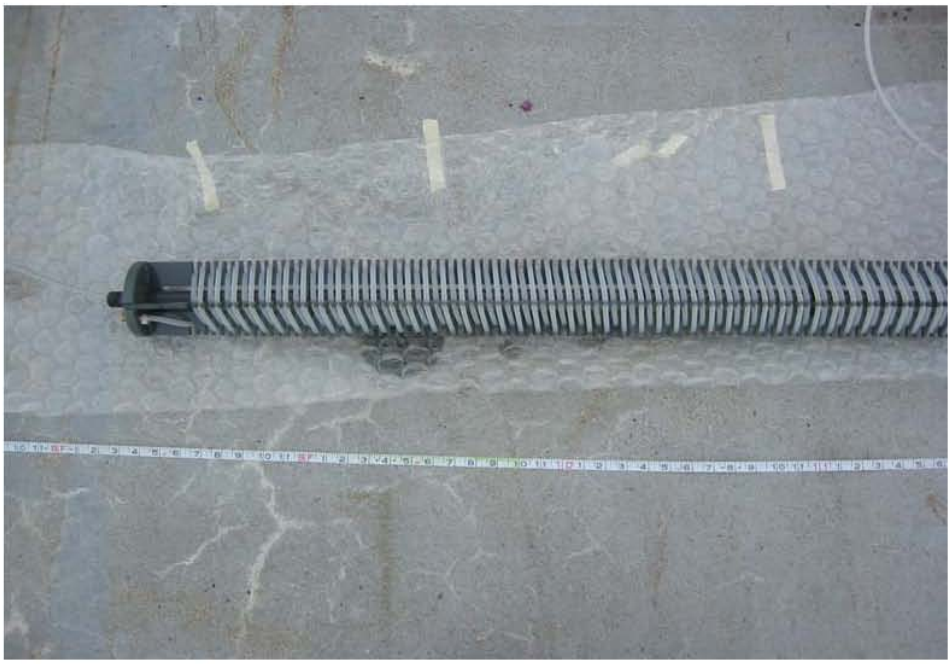
Down-well Waterloo Emitters (Figure 5-6) were deployed in injection wells IW-01 and IW-04 to promote aerobic conditions within Plots #1 and #4. The emitters consisted of silicone tubing coiled around a 4-ft long PVC frame. Two emitters were joined together in each well to target the majority of the screened interval. Each series of emitters was connected to an air cylinder and pressure regulating valve, which provided a constant supply of oxygen to the emitters. The air cylinders and regulating valves were housed within the protective well vaults (Figure 5-6). Compressed air was used instead of compressed oxygen as JS666 is sensitive to oxygen levels greater than 10 mg/L.

5.4 FIELD ACTIVITIES

Field activities following well installation consisted of buffer injections, aeration, and bioaugmentation. Two bioaugmentations were performed during the demonstration: one in October 2008 and one in February 2009. The monthly field events consisted of groundwater sampling and buffer injections, with the exception of the final field event (May 2009) where only groundwater sampling was conducted. The Gantt Chart presented in Figure 5-7 outlines the schedule for each monthly sampling and buffer injection event. Specifics of the field operations are discussed in the sections below with the exception of field calibration procedures, quality assurance sampling, decontamination practices, and sample documentation, which are described in Appendix D.


120.0
100.0
80.0
60.0
40.0
20.0
0.0
Iodide Concentration (mg/L)
Tracer Added
September 20, 2009
19-Sep-08
21-Sep-08
23-Sep-08
25-Sep-08
27-Sep-08
29-Sep-08
1-Oct-08
3-Oct-08
5-Oct-08
7-Oct-08
Date
Legend
MW-01 Field Probe
MW-03 Field Probe
MW-02 Field Probe
MW-02 Lab Data
DRAFT
Iodide Tracer Test, Bioaugmentation Plot 1
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Figure
5-4
Guelph
October 2009


Bromide Concentration (mg/L)
Precipitation (inches)
Date
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
19-Sep-08
21-Sep-08
23-Sep-08
25-Sep-08
27-Sep-08
29-Sep-08
1-Oct-08
3-Oct-08
5-Oct-08
7-Oct-08
Legend
MW-04 Field Probe
MW-06 Field Probe
MW-05 Field Probe
MW-05 Lab Data
Precipitation
DRAFT
Bromide Tracer Test, Bioaugmentation Plot 2
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Figure
5-5
Guelph
October 2009



Waterloo Emitter

Compressed Air Cylinder

Waterloo Emitter and Compressed Air Cylinder
Site 21, St. Julien's Creek Annex, Chesapeake, VA

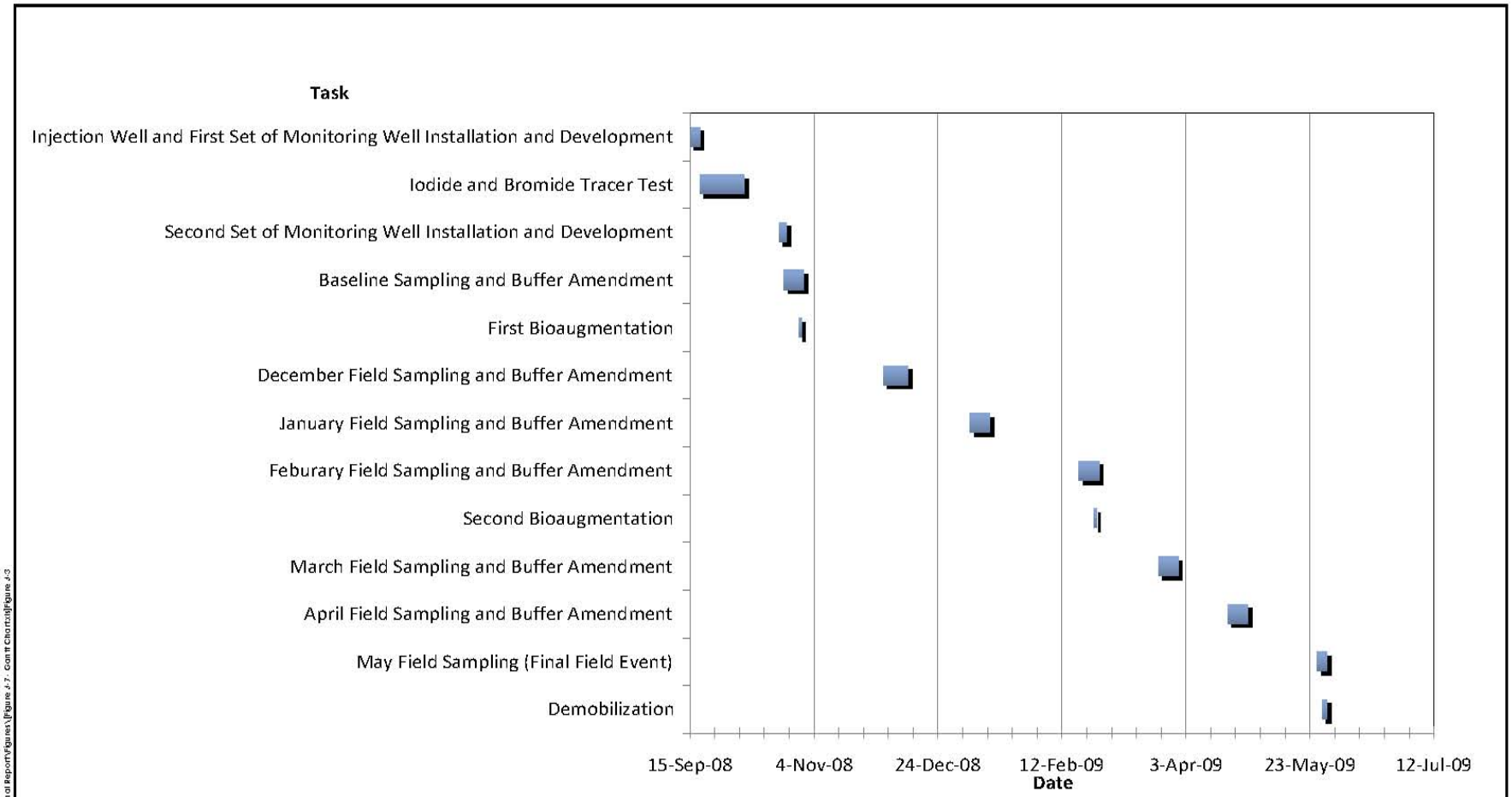
DRAFT

Geosyntec consultants

Guelph | September 2009

Figure 5-6

P:\PRJ\Projects\TR0207-BSCP JSA66 Boeing\Task 7- Reporting\Final Report\figures\figure 5-6 - emitterphotos.ai[figure 5-6


Task
Injection Well and First Set of Monitoring Well Installation and Development
Iodide and Bromide Tracer Test
Second Set of Monitoring Well Installation and Development
Baseline Sampling and Buffer Amendment
First Bioaugmentation
December Field Sampling and Buffer Amendment
January Field Sampling and Buffer Amendment
Feburary Field Sampling and Buffer Amendment
Second Bioaugmentation
March Field Sampling and Buffer Amendment
April Field Sampling and Buffer Amendment
May Field Sampling (Final Field Event)
Demobilization
15-Sep-08
4-Nov-08
24-Dec-08
12-Feb-09
3-Apr-09
23-May-09
12-Jul-09
Date
DRAFT
Schedule of Field Events
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Guelph
October 2009
Figure
5-7

5.4.1 Buffer Amendments and Aeration

A phosphate buffer consisting of potassium monobasic orthophosphate (KH_2PO_4) and potassium dibasic orthophosphate (K_2HPO_4) was added monthly to the injection well of each test plot to raise the groundwater pH to 7.1-7.2, as JS666 loses its activity below a pH of 6.5. Groundwater from each injection well was extracted, amended with the phosphate buffer, and re-injected. All pumps, tubing, and tanks were dedicated for each plot to prevent cross-contamination. Table 5-2 lists the volume of groundwater amended and amount of buffer added to each test plot. The volume of buffer was increased during the February field event to provide additional fluid to distribute the JS666 during the second bioaugmentation.

Air was added to the injection wells in Bioaugmentation Plot #1 and Control Plot #4 using Waterloo Emitters, down-well diffusers described in Section 5.3.3. Two four foot emitters were connected in series and placed in the injection wells IW-01 and IW-04 and positioned so they spanned the full length of the well screens. The silicone tubing in the emitters was pressurized to between 10 and 15 psi with the air canister to allow for the diffusion of oxygen into the groundwater.

The emitters were removed during the sampling of their respective injection wells and, at the end of each field event, the emitter discharge tubing needle valves were opened for 5-7 seconds to purge the air in the silicone tubing. Before the emitters were returned to the injection wells, the silicone tubing and connections were checked for leaks.

5.4.2 Bioaugmentation #1

The first bioaugmentation was planned for October 29, 2008. The specific activity of the culture was measured over 3-4 hours in 50 mL samples collected from the 64-L reactor prior to injection and specific activities were measured directly in the 64-L reactor over 24 hours prior to delivery of the culture to the site. Specific activity in 50 ml samples was calculated from the difference in cDCE measured in serum bottles by gas chromatography over time, normalized to soluble protein (Pierce BCA Protein Assay Kit). 24 hour rates were calculated from the total volume of cDCE consumed by the reactor in the 24 hours prior to harvest, normalized to soluble protein. Table 5-3 provides the specific activities prior to the first bioaugmentation, which are in the vicinity of the maximum rates of 12.6 – 16.8 nmol-cDCE • min^{-1} • mg-$protein^{-1}$ reported by Coleman *et al.* (2002).

The culture was delivered to the site on the same day as the bioaugmentation, October 29, 2008. Approximately 8 L of culture (density of 1.8×10^9 colony forming units [cfu]/mL according to optical density [O.D.] measurements) were added to each of the test plots following the injection of 500 L of buffer. Then, the remaining 1500 L of buffer were injected.

TABLE 5-2: Buffer Amendments
Site 21, St. Julien's Creek Annex, Chesapeake, VA

Location	IW-01	IW-02	IW-03	IW-04
Baseline Buffer Addition (During First Bioaugmentation)				
Volume of Water Removed (gal)	530	530	530	530
KH_2PO_4 - Monobasic (g)	3,400	3,400	3,400	3,400
K_2HPO_4 - Dibasic(g)	4,400	4,400	4,400	4,400
December, January, March, and April Buffer Addition				
Volume of Water Removed (gal)	160	235	235	160
KH_2PO_4 - Monobasic (g)	581	872	872	581
K_2HPO_4 - Dibasic(g)	1,870	2,800	2,800	1,870
February Buffer Addition (During Second Bioaugmentation)				
Volume of Water Removed (gal)	246	370	370	246
KH_2PO_4 - Monobasic (g)	900	1,356	1,356	900
K_2HPO_4 - Dibasic(g)	2,896	4,360	4,360	2,896

Notes:
gal - gallons
g - grams

5.4.3 Aeration of Buffer

From February 2009 onward, the extracted groundwater from all four plots was oxygenated to a DO concentration just below 10 mg/L to promote biodegradation. After the buffer amendments were added to the extracted groundwater in the 600 gal and 300 gal storage tanks, a flat air diffusion stone was connected to an oxygen tank and regulator using a length of polyethylene tubing and lowered to the bottom the each tank. A YSI multi-parameter meter was placed just under the surface of the water in the tank. The oxygen was allowed to flow through the diffusion stone until the DO concentration of the extracted groundwater reached the desired concentration. Dedicated aeration equipment was used for each plot's tank.

5.4.4 Bioaugmentation #2

Because the pH was not optimal after the first bioaugmentation, the activity of the bacteria was not as high as desired. Consequently, a second bioaugmentation was planned for February 25, 2009. The second culture was grown from cells that had been frozen at -80°C on 19 September. Pure cDCE was provided to the culture from the time the frozen cells were placed in the reactor on 17 February until harvest on 24 February. The specific activity of the culture was measured in 50 mL aliquots over 3-4 hours from samples collected prior to injection and specific activities were measured in the 64-L reactor over 24 hours prior to delivery to the site (Table 5-3).

The culture was delivered to the field site on 25 February, 2009. For this bioaugmentation, 9 L of culture (density of 2.3×10^9 cfu/mL based on O.D. measurements) were injected into each bioaugmentation plot. 4.5 L of culture was first dispersed in 1400 L of buffer; 150-300 L of buffer with culture was first injected, then the remaining 4.5 L of culture was co-injected directly into the well, followed by the remaining buffer/culture solution. This approach was taken in an attempt to improve the distribution of the bacteria in the subsurface.

5.4.5 Shut-down/Demobilization

Site demobilization followed the final field sampling event. The demobilization included the removal of the Waterloo Emitters from IW-01 and IW-04, removal of all dedicated non-reusable field equipment including the 600 gal and 300 gal water tanks by Capitol Environmental Services of Roanoke, Virginia (Capitol Environmental), and the removal of the portable toilet and storage container (which was used for on-site storage of sampling equipment and supplies between sampling event) by their appropriate vendors. Section 5.4.6 outlines the removal and disposal of the non-hazardous site derived soil and purge water (IDW waste). Following the removal of the IDW waste, the IDW secondary containment was dismantled and removed by Capitol Environmental. The Navy elected to adopt the management of the demonstration wells as an alternative to decommissioning.

TABLE 5-3: Specific Activity of JS666 Prior to Bioaugmentation
Site 21, St. Julien's Creek Annex, Chesapeake, VA

Culture	Date	OD_{600}	Protien (mg/mL)	Specific Activity (nmol-cDCE•min^{-1}•mg-$protien^{-1}$)
October, 2008	27-Oct-08	1.006	0.113	10.05
	27-28 Oct, 2008 (24 hour)			10.4
	28-Oct-08			17.04
February, 2009	23-Feb-09	0.827	0.08	15.67
	23-24 Feb, 2009 (24 hour)			17.16

Notes:
cDCE - cis-1,2-dichloroethene
mg/mL - milligrams per milliliter
mg-protien - milligrams of protien
min - minutes
nmol - nanomol
OD600 - optical density at 600 nanometers

5.4.6 Disposal of IDW

All soils and water generated during well installation, well purging, and equipment cleaning were containerized by the drilling or sampling personnel in approved Department of Transportation (DOT) 55-gal drums. The drums were sealed and transported to a designated storage area where they were properly labeled and stored on secondary containment, as appropriate. The drums were sampled for disposal characterization and classified as non-hazardous waste. Based on the analytical results, the non-hazardous waste was disposed at an approved disposal facility as authorized by the Navy. The waste was removed from the site and transported to the approved disposal facility by Capitol Environmental. All other common, non-hazardous trash associated with the demonstration was disposed of according to SJCA protocols or removed and disposed of by Capitol Environmental at the end of the demonstration.

5.5 GROUNDWATER SAMPING

5.5.1 Parameters and Frequency

Prior to the injection of any amendments, groundwater samples were collected from each of the demonstration wells to determine baseline concentrations. A description of the samples collected during each phase of the project, the number and type of samples collected, and the rationale are presented in Table 5-4. In addition, water levels were collected monthly prior to sampling each well to help identify any changes in the direction of groundwater flow. All water level measurements were obtained using a battery-operated water level tape marked at a minimum of 0.01 ft intervals.

Following injection of the JS666 culture, groundwater samples were collected from the demonstration wells for the following measurements and analyses:

- VOCs, alkalinity and dissolved metals approximately once every month for 6 months;
- Dissolved Hydrocarbon Gases (DHG) approximately every 2 months for 6 months;
- JS666 activity using microcosm activity assays approximately once every month for 6 months;
- JS666 detection using molecular probes approximately once every month for 6 months;
- cDCE carbon isotope samples approximately once every month for 6 months; and
- Water levels approximately once every month for 6 months or as required;

5.5.2 Groundwater Sampling Method and Sample Preservation

Prior to collecting groundwater samples for chemical analysis, the stagnant water in the well casing was purged using dedicated Waterra® pumps to allow sampling of groundwater that was

TABLE 5-4: Total Number and Types of Samples Collected
Site 21, St. Julien's Creek Annex, Chesapeake, VA

Component	Analyte	Specific Parameter of Interest	Frequency	Number of Samples	Location	Rationale/Use
Pre-demonstration sampling	Tracer Test	bromide, iodide	Every 4 to 6 hours or twice daily	227	IW-01, IW-02, MW-01, 02, 03, 04, 05, and 06	Assess direction and rate of groundwater flow and confirm positioning of monitoring wells
	Field Parameters	DO, ORP, pH, conductivity, temperature	Once	4	All injection wells prior to VOC sampling	Primarily to monitor significant shifts in redox conditions and pH
	VOCs	TCE, cDCE, tDCE, 1,2-DCA, VC	Once	4	All injection wells	Assess the extent of degradation occurring in the bioaugmentation plots relative to the control plots
Demonstration Sampling	Field Parameters	DO, ORP, pH, conductivity, temperature	Baseline and monthly for 6 months	184	All injection and monitoring wells prior to sampling	Primarily to monitor significant shifts in redox conditions and pH
	VOCs	TCE, cDCE, tDCE, 1,2-DCA, VC	Baseline and monthly for 6 months	192	All injection and monitoring wells	Assess the extent of degradation occurring in the bioaugmentation plots relative to the control plots
	DHGs	methane, ethane, ethene	Baseline and monthly to once every 2 months	96	All injection and monitoring wells	Assess whether anaerobic degradation processes are occurring due to inefficient distribution of oxygen
	Alkalinity	calcium carbonate	Baseline and monthly for 6 months	168	All injection and monitoring wells	Monitor increases in alkalinity due to microbial activity
	Dissolved Metals	Fe^{2+}, Mn^{2+}	Baseline and monthly for 6 months	168	All injection and monitoring wells	Monitor major shifts in redox conditions
	cDCE Carbon Isotopes	^{13}C, ^{12}C	Once during baseline sampling; once at end of demonstration	168	All injection and monitoring wells	Changes in $^{13}C/^{12}C$ fraction of cDCE are used to assess biological transformation of cDCE as it migrates through the test plots
	JS666 Activity	NA	Baseline and monthly for 6 months	168	All injection and monitoring wells	Assess the distribution (transport) of JS666 within the test plots
	JS666 Detection	NA	Baseline and monthly for 6 months	168	All injection and monitoring wells	Assess the distribution (transport) of JS666 within the test plots

Notes:
NA - Not Applicable

representative of aquifer conditions. The Waterra® pump system consisted of a Delrin® foot-valve attached to rigid, 5/8-inch outside diameter, high-density polyethylene (HDPE) tubing equal in length to the depth of the well. Oscillation of the tubing, together with the action of the foot valve, forced water to the ground surface (i.e., inertial pump). The entire pump assembly was dedicated to the well, reducing the potential for cross-contamination between wells. Appendix D contains information on the Waterra® pumps.

The Waterra® pumps were placed at the mid-point of the well screen, and pumped at a rate of <1 gallons per minute (gpm). The water level in the well was measured immediately before purging started and immediately after purging. Measurements of field parameters (DO, ORP, pH, conductivity, and temperature) were conducted during well purging using a YSI 556 MPS or YSI 600XL multi-parameter meter and flow-through cell. Groundwater samples were not collected until ORP measurements of the purged water had stabilized to within about 10% of the previous reading, or until three well casing volumes has been purged.

Groundwater samples collected for laboratory analysis were transferred directly from the pump tubing into the sample containers provided by the laboratory performing the analyses. Sample containers (40 mL glass volatile organic analysis [VOA] vials) for VOCs, DHGs, and cDCE isotopes containing concentrated hydrochloric acid (HCl; pre-added to sample bottles by the laboratory to produce a sample pH <2) and were filled with minimal turbulence and without headspace in the container. As a VOC preservative, HCl extends the holding time of the samples from 7 to 14 days and inhibits microbial degradation of the VOCs. A minimum of two containers were filled for each VOC and DHG sample, while a minimum of 7 containers were filled for each cDCE isotope sample. Samples for dissolved metals were pumped through a disposable in-line filter (0.45 μm nominal pore size) prior to being collected in the sample container. All dissolved metals samples were collected in HDPE containers and preserved using concentrated nitric acid (pre-added to sample bottles by the laboratory to produce a sample pH <2). Samples for tracers, alkalinity, and microbial (JS666 activity and detection) analyses were collected using 120 mL, 250 mL, 1 L, and 120 mL HDPE containers, respectively, and were not chemically preserved.

5.6 ANALYTICAL METHODS

The analytical methods used to analyze groundwater samples are presented in Table 5-5. In the following sections, the methodology for the isotope analyses, microcosm activity assays, and probe assays are described. Isotope analyses were conducted at the University of Toronto and microcosm and probe assays were conducted at Cornell University. Information pertaining to calibration of analytical equipment, quality assurance, decontamination, and sample documentation can be found in Appendix D.

5.6.1 Isotopic Analyses

Compound specific isotope analysis (CSIA) measures the ratio of heavy and light elements ($R=^{13}C/^{12}C$) in a compound and is compared to an international standard (V-PDB for carbon):

TABLE 5-5: Summary of Sample Handling and Laboratory Analytical Details
Site 21, St. Julien's Creek Annex, Chesapeake, VA

Parameter	Analytical Method	Method Number	Analytical Laboratory	Quantitation/Reporting Limit[1]	Sample Container	Preservative	Holding Time
Field Parameters (DO, ORP, pH, conductivity, temperature)	Field probes	Field	NA	Varies	NA	NA	NA
VOCs (TCE, cDCE, tDCE, 1,2-DCA, VC)	Gas Chromatography /Mass Spectrometry	EPA 8260B	CAS	1 - 20 µg/L	3 x 40 mL VOA	HCl to pH <2, cool to <6°C	14 days
Dissolved Hydrocarbon Gases (methane, ethane, ethene)	Gas Chromatography/ Flame Ionizing Detector	RSK-175	CAS	1-2 µg/L	3 x 40 mL VOA	HCl to pH <2, cool to <6°C	14 days
Tracers (bromide, iodide)	Ion-Selective Electrode	Field	NA	0.005-0.4 mg/L	120 mL plastic	cool to <6°C	28 days
Alkalinity	Titration	EPA 310.1, SM 2320B	CAS	2-40 mg/L	250 mL plastic	cool to <6°C	14 days
Dissolved Metals (Fe^{2+}, Mn^{2+})	Inductively-Coupled Plasma	EPA 6010B	CAS	0.01-0.1 mg/L	250 mL plastic	filter on-site, HNO_3 to pH <2	180 days
cDCE Carbon Isotopes (^{13}C, ^{12}C)	Gas Chromatography/ Combustion/ Isotope Ratio Mass Spectrometry	NA	U of T	10 µg/L	8 x 40 mL VOA	1mL 12N HCl, cool	NA
JS666 Activity	Microcosm Activity Assay	NA	Cornell	0.5% loss of cDCE per day	2 x 1 L plastic*	cool to 4°C	14 days
JS666 Detection	Molecular Probe	NA	Cornell	3,000 copies/mL	120 mL plastic*	cool to 4°C	14 days

Notes:
NA - Not Applicable
CAS - Columbia Analytical Services, Inc., Rochester, NY
U of T - University of Toronto Stable Isotope Lab, Toronto, ON
Cornell - Cornell University, Ithaca, NY
[1] - Quantitation/reporting limit for undiluted sample
*sterile (or from new, unused stock)

$$\delta^{13}C_{compound} = ((R_{compound} / R_{standard}) - 1) \times 1000 \qquad \text{Eq (1)}$$

where the $\delta^{13}C$ value is expressed in permil (‰) units.

Carbon isotopic values for chlorinated ethenes were measured by purge and trap. These samples were injected onto a Velocity XP purge and trap (Teledyne Tekman) equipped with a Vocarb 3000 trap. The purge time was 11 min at 23°C followed by 1 min dry purge with 40 mL/min Helium flow, and desorption for 2 min at 250°C with 50 mL/min flow. The analyte was transferred onto a Varian 3400 GC fitted with a VOCOL column (30 m x 0.25 mm inside diameter; Chrompack; flow 1.3 mL/min) for cDCE isotope measurements. The purge water volume was 10 mL and the split varied between 6:1 to 300:1. The GC temperature program for cDCE commenced at 35°C and increased at a rate of 2°C per minute to 55°C, then increased at a rate of 10°C per minute to 90°C. The GC was interfaced with a combustion oven and a Finnigan MAT Delta Plus XL mass spectrometer. The combustion oven consisted of a copper oxide, platinum and nickel oxide wire held at 980°C. The chlorinated ethene was oxidized in the oven to CO_2 and water. The water was removed via a Nafion™ membrane water trap and the CO_2 entered the mass spectrometer for isotopic analysis (Chartrand, 2007). The analytical uncertainty of carbon isotopic measurements is ±0.5 ‰ (Sherwood Lollar *et al.*, 2007), which incorporates both the accuracy of the measurement with respect to international standards and the reproducibility on replicate measurements of the sample. cDCE isotopic standards run throughout each experiment agreed with laboratory working standards.

For aerobic cDCE biodegradation, the extent of fractionation during the initial transformation step of a contaminant can be described using the Rayleigh model equation:

$$R/R_0 = f^{(\alpha-1)} \qquad \text{Eq (2)}$$

where R is the isotopic measurement ($^{13}C/\ ^{12}C$) of the substrate at any given fraction remaining (f), R_0 is the initial isotopic ratio and α is the fractionation factor. The fractionation factor is a measure of the extent of fractionation occurring during a reaction, and if the reaction fits a Rayleigh model, α remains constant throughout the reaction (Mariotti *et al.*, 1981). The fractionation factor can also be expressed as an enrichment factor (ε, in ‰), which is calculated by:

$$\varepsilon = 1000\ (\alpha - 1) \qquad \text{Eq (3)}$$

5.6.2 Microcosm Assays

Microcosm Preparation

Groundwater samples were shipped via overnight express courier (packed in ice) from the field site to Cornell University; they were stored at 4˚C until used for preparation of microcosms (within one week of arrival). All microcosms were prepared in triplicate and consisted of 50 mL

groundwater samples (transferred aseptically with sterilized pipettes) in autoclaved, 160 mL glass serum bottles fitted with autoclaved, Teflon®-lined, butyl-rubber septa and aluminum crimps. After samples had been transferred to microcosms, the remainders were used for pH measurement.

Because it was anticipated that native cDCE levels in samples might be very low, particularly as the study progressed in bioaugmented test plots, each microcosm had 0.5 µmol cDCE aseptically added from a sterile, aqueous stock solution. This addition resulted in a nominal concentration (i.e., ignoring partitioning to headspace) to each microcosm of approximately 1 mg/L cDCE, insuring a cDCE level sufficient for activity assays, without resulting in an order-of-magnitude increase over existing native levels. All aseptic transfers (injections to and sampling from bottles) were through ethanol-swabbed flamed septa. Microcosms were incubated at 22ºC in the dark, agitated at 160 RPM.

Microcosm Analysis

Total quantities of cDCE, VC, and TCE in bottles were measured from 250-µL headspace samples by gas chromatography (Perkin-Elmer, Autosystem GC) with a flame-ionization detector and a packed column (1% SP-1000 on 60/80 Carbopack B [Supelco]). Headspace samples were aseptically acquired with sterile needles through flamed microcosm septa, using the syringe for sample withdrawal. Levels were quantified (coefficient of variation of 4 to 7%) through comparison to standard curves created from known additions to replicate serum bottles containing dH_2O. Detection limits for cDCE, VC, and TCE were approximately 10 µg/L, 1 µg/L, and 3 µg/L, respectively. Microcosms were analyzed for approximately 40 days, with more frequent sampling during the first week. Sampling alternated among triplicates.

5.6.3 Molecular Probe Assays

Sampling Procedure and Nucleic Acid Extraction

Groundwater samples were shipped via overnight express courier (packed in ice) from the field site to Cornell University; they were stored at 4˚C until extracted, which occurred within one week of arrival. DNA was extracted (in duplicate) from 0.5-mL groundwater samples using the UltraClean Soil DNA Kit (MoBio, Carlsbad, CA). All DNA extractions were stored at -20ºC until later analysis (typically within one week of extraction).

Quantitative Polymerase Chain Reaction (qPCR)

The DNA, and therefore the number of target genes in each sample, were quantified by real-time, quantitative polymerase chain reaction (qPCR) with a thermocycler (iCycler Detection System, BIO RAD) with the intercalation culture iQ SYBR Green (BIO RAD). The reactions were carried out under the following conditions: 2 min at 50ºC followed by 3 min at 95ºC; next 40 cycles (denaturation at 95ºC for 15 sec, annealing and extension at 63ºC for 1 min), where fluorescence was measured after every cycle. Each reaction was performed in triplicate (which,

given that there were duplicate extractions of each water sample, meant there were six reactions conducted per water sample), and a melt curve was completed following the amplification reactions to confirm the specificity of the primers and the reactions. Reactions that produced PCR products with unusual melt curves were disregarded (i.e., not considered positive for the probe target).

qPCR Application to SJCA Samples

To overcome soil-matrix inhibition to the PCR reaction, DNA extracts were diluted. To determine the minimum level of dilution required, the following procedure was performed. Pure-culture JS666 was used to inoculate several different samples, and DNA extractions were performed. Extracted DNA was diluted 1-, 5-, 10-, 20-, 50-, 100-, and 200-fold and the copy-number was measured using qPCR. These were compared against the expected amount of DNA as determined by an extraction performed on the inoculum culture. The minimum dilution required was found to be only 5-fold, which became the dilution level used throughout the study.

Expression Data Analysis (DART)

To damp-out errors associated with plate-to-plate variation in standard curves, fluorescence data generated by the iCycler was analyzed using the DART-PCR technique as outlined and developed by Peirson *et al.* (2003). The DART-PCR tool uses linear regression to calculate an initial fluorescence level, R0, in each well (Schefe *et al.*, 2006). The JS666 standard conversion factor between initial fluorescence (R0) and ng of DNA per reaction was created for qPCR. This number, in units of R0/ng DNA, was found by averaging pure JS666-DNA samples of known concentrations of standard curves. Measured concentration of DNA was converted to copies of target gene per microliter of sample (Equations 1 and 2), where the size of the JS666 genome is 5.9 Mb as reported by the Joint Genome Institute Microbial Sequencing Program. The total mass of DNA (grams) per mole of JS666 cells is thus given by

$$\left(\frac{g\ DNA}{mol}\right) = \left(5.9\times10^{6}\ \ bp\right)\times 660\left(\frac{daltons}{bp}\right) \qquad \text{Eq (4)}$$

and therefore the number of copies per µL is found by

$$\left(\frac{copies}{\mu l}\right) = \frac{6.02\times10^{23}\left(\frac{copies}{mol}\right)\times C_{DNA}\left(\frac{g}{\mu l}\right)}{\left(\frac{g\ DNA}{mol}\right)} \qquad \text{Eq (5)}$$

where C_{DNA} is the concentration of DNA as measured by Fluoroskan

To quantify the total concentration of DNA from pure culture with fluorometry, the intercalating reculture PicoGreen (Invitrogen) was employed. A Fluoroskan Ascent

spectrophotometer (Thermo Labsystems) measured fluorescence of PicoGreen bonded to double-stranded DNA at an excitation wavelength of 485 nm and emission wavelength of 538 nm. Lambda DNA (Invitrogen) was used as a primary standard. Since DNA in extracts from pure JS666 cultures was assumed to be entirely comprised of JS666 DNA, the DNA concentrations of JS666-DNA stocks were determined by applying the lambda DNA standard curve.

The conversion number between iCycler fluorescence and target gene copy number was found for the isocitrate lyase, the cyclohexanone monooxygenase, and 16S rRNA genes by averaging 13 pure JS666-DNA samples of known concentrations (as determined with the Fluoroskan).

In practice, the minimum quantifiable concentration level from all probes was judged to be that which corresponds to 20 target-gene copies per PCR reaction (copies/rxn), and therefore about 3 x 10^3 copies/mL of original sample. When replicate plate-wells agreed among themselves at lower than 20 copies/rxn we considered the test positive for the target, but we did not attempt to report quantitative information unless there were greater than 20 copies/rxn.

As for pre*cis*ion, our studies indicate a coefficient of variation (CV = 100*std dev/mean) of ± 43%. This CV value includes not only the impre*cis*ion of the qPCR procedure itself, but also of the DNA extraction and comparison across plates. About one-half of the overall variance occurred in the extraction step; estimates of the CV in total DNA as determined by fluorometry on replicate extractions was only about ± 20%. The estimates of initial copy-numbers of replicate samples of pure JS666 DNA had intra-run (within plate) CVs of 0.6 -1.5%, whereas the inter-run (across plates) CV was ± 21%. These results suggest that simply comparing samples across qPCR plates accounts for approximately one-half of the variance seen in the overall process.

5.7 SAMPLING RESULTS

In the following sections, the analytical results are summarized. Water level elevation data can be found in Section 5.7.1, Field Parameters in Section 5.7.2, Geochemical Parameters in Section 5.7.3, a summary of the Isotopic Analyses in Section 5.7.4, Volatile Organic Compound data in Section 5.7.5, and Microcosm Assay and Probe Assay Results in Section 5.7.6, and. A complete compilation of the analytical data is presented in Appendix E. All data were validated using USEPA data qualifiers for organic and inorganic data (USEPA 540-R-08-01 and 540-R-04-004). A summary of the data validation results and findings is presented in Appendix G.

5.7.1 Water Level Elevation Data

Water levels were collected prior to sampling each well to help identify any changes in the direction of groundwater flow. A complete compilation of measured water level elevations is presented in Appendix C. However, due to the limited amount of data collected and because of the close proximity of the wells, groundwater flow directions could not be confidently predicted. As a result, field parameter (pH and specific conductivity) data for each sampling event was used

to assess groundwater flow directions. Estimated groundwater flow directions are shown on the various VOC, field parameter, geochemical, and qPCR/Microcosm Assay figures referenced below.

5.7.2 Field Parameters

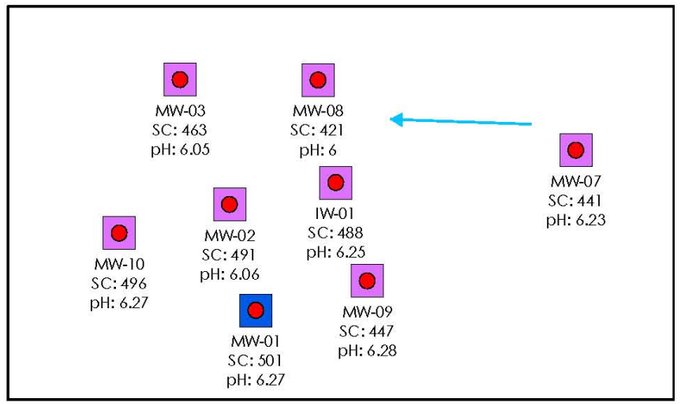
Following the initial buffer injections, increases in groundwater pH and specific conductivity (0.11 to 0.69 pH units and 148 to 1001 µS/cm, respectively, as measured during the December sampling event) were observed in the injection wells and immediately downgradient monitoring wells for all plots (Figures 5-8a to 5-8d). Slight increases in these parameters were also observed in a few of the transgradient monitoring wells in the bioaugmentation plots. For all plots, the increased pH and specific conductivity levels were generally sustained throughout the project duration as a result of continued buffer injections. However, buffer injections did not appreciably impact the pH in MW-09 and MW-01 in Bioaugmentation Plot #1 and MW-13 and MW-14 in Bioaugmentation Plot #2. No significant changes in pH were observed in the upgradient wells for either bioaugmentation plot.

In Plots #1 and #4, groundwater ORP and DO concentrations increased significantly in injection wells IW-01 and IW-04 (which were both equipped with oxygen emitters) throughout most of the demonstration (Table E-4 in Appendix E). In IW-01, dissolved oxygen levels increased from 0.53 mg/L to levels generally above 2.97 mg/L, and ORP levels increased from 24.7 mV to levels generally above 100 mV (during the April and May sampling events it was discovered that the air cylinder for the oxygen emitter had prematurely emptied, thus resulting in decreased DO levels during these events). However, the DO increases were predominately limited to the injection wells themselves. DO levels in the downgradient wells remained relatively unchanged, with concentrations ranging from 0.08 mg/L to 1.24 mg/L for all sampling events except the April event, where DO levels were slightly elevated in most downgradient wells but also elevated in the upgradient well, MW-07. ORP levels in the downgradient wells ranged between -382.2 mV and 34.8 mV.

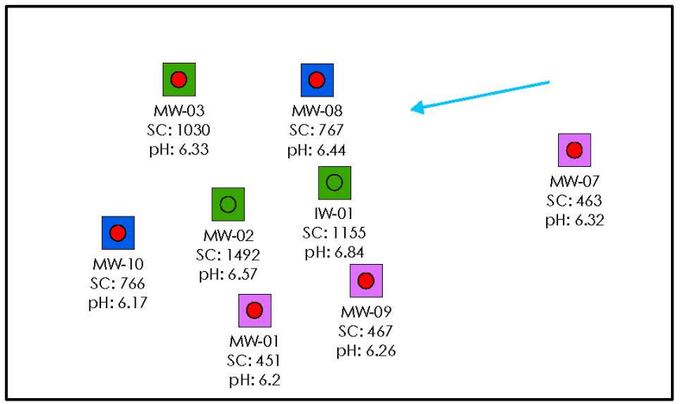
In Plots #2 and #3, increases in both DO and ORP were observed in wells IW-02 and IW-03 only immediately following buffer injection (likely because of elevated DO concentrations in the injected buffers as a result of mixing and/or aeration during buffer preparation). By the following event DO and ORP had returned to pre-buffer injection levels, which ranged from 0.17 mg/L to 0.57 mg/L and -376.7 mV and 162.1 mV, respectively. In the downgradient wells, DO concentrations were generally less than 1 mg/L, and ORP levels were predominately negative.

5.7.3 Geochemical Parameters

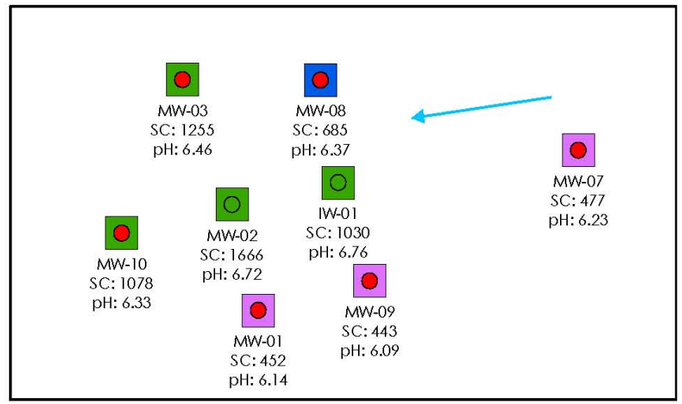
Throughout the study duration, significant increases in groundwater alkalinity were observed in all plots (Figures 5-9a to 5-9d). Increases in alkalinity were predominately observed in wells immediately downgradient of the injection wells, with smaller increases in the



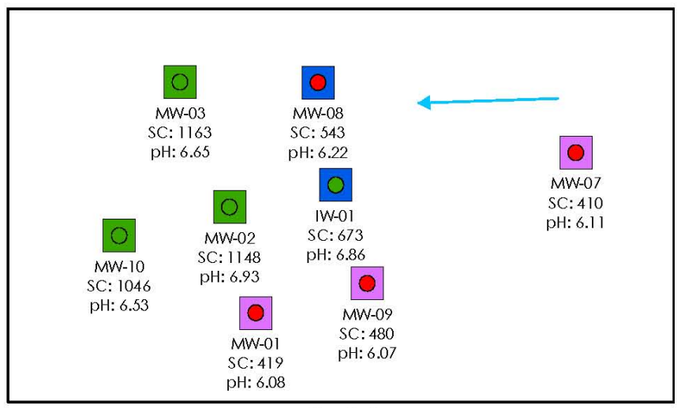
Baseline
October 2008



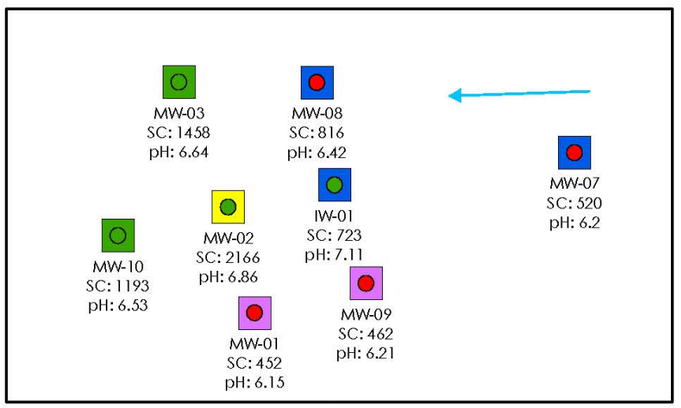
Month 1
December 2008



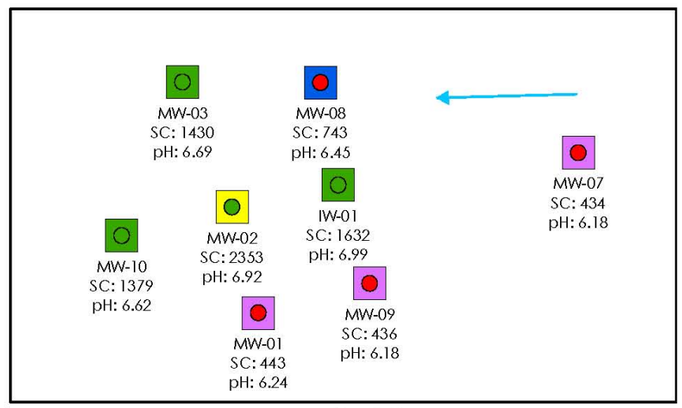
Month 2
January 2009



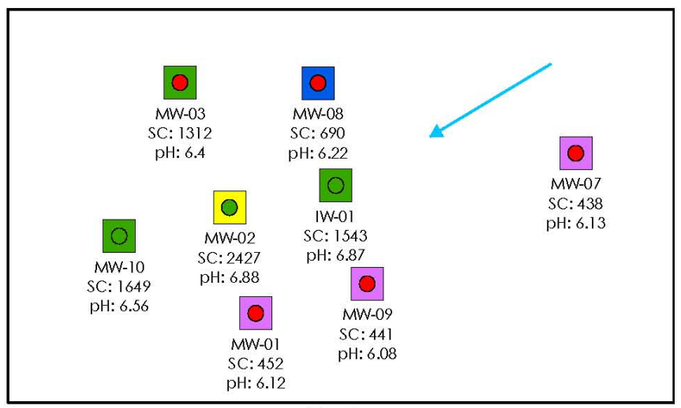
Month 3
February 2009



Month 4
March 2009



Month 5
April 2009



Month 6
May 2009

Legend

Specific Conductance (uS/cm)

- ≤ 500
- > 500 - 1,000
- > 1,000 - 2,000
- > 2,000

pH

- ≤ 6.5
- > 6.5 - 8
- > 8

general direction of groundwater flow

4 2 0 4 Feet

Specific Conductance and pH for Plot #1

Site 21, St. Julien's Creek Annex, Chesapeake, VA

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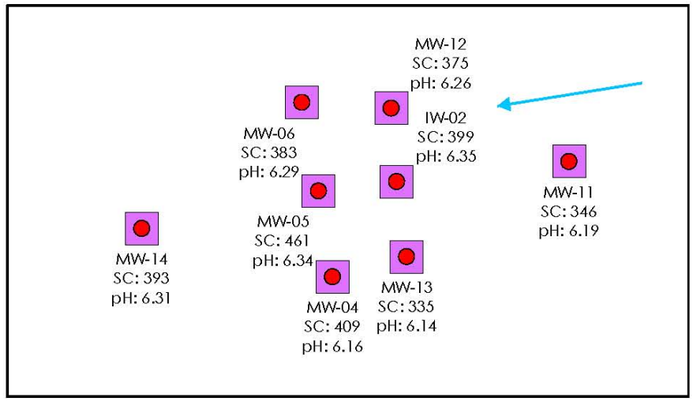
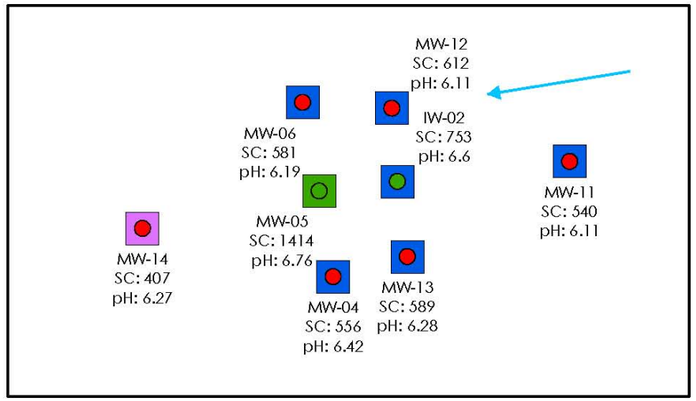
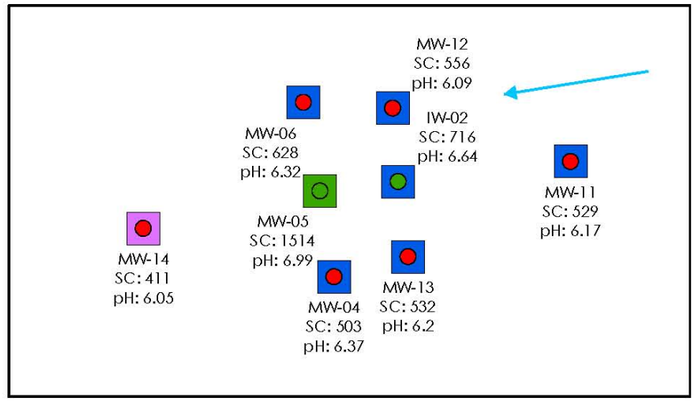
Figure **5-8a**



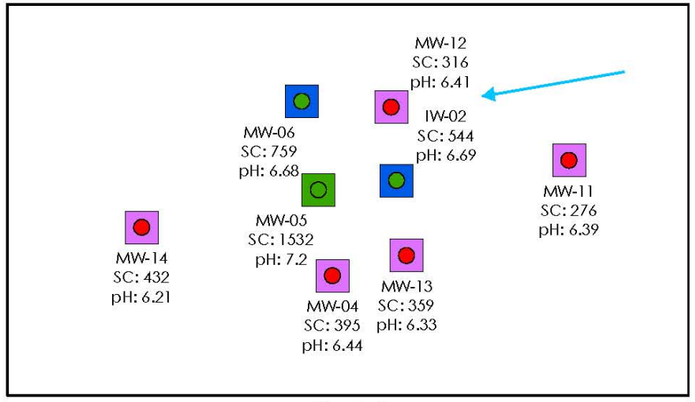
Baseline
October 2008



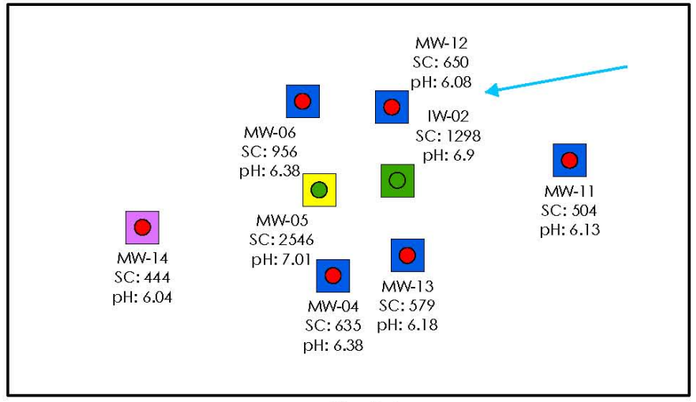
Month 1
December 2008



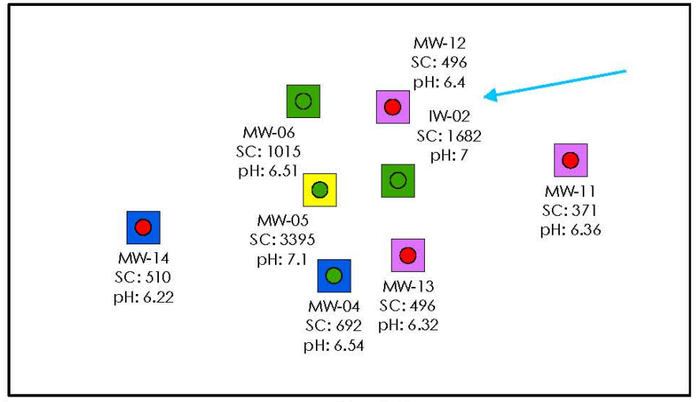
Month 2
January 2009



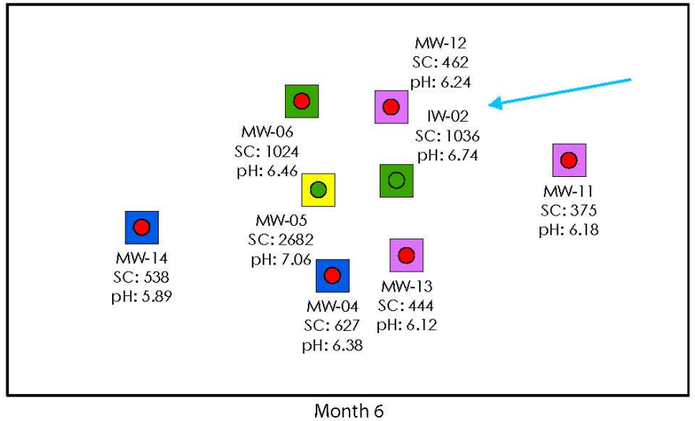
Month 3
February 2009



Month 4
March 2009



Month 5
April 2009



Month 6
May 2009

Legend

Specific Conductance (uS/cm)

- ≤ 500
- > 500 - 1,000
- > 1,000 - 2,000
- > 2,000

pH

- ≤ 6.5
- > 6.5 - 8
- > 8

general direction of groundwater flow

N

4 2 0 4 Feet

Specific Conductance and pH for Plot #2

Site 21, St. Julien's Creek Annex, Chesapeake, VA

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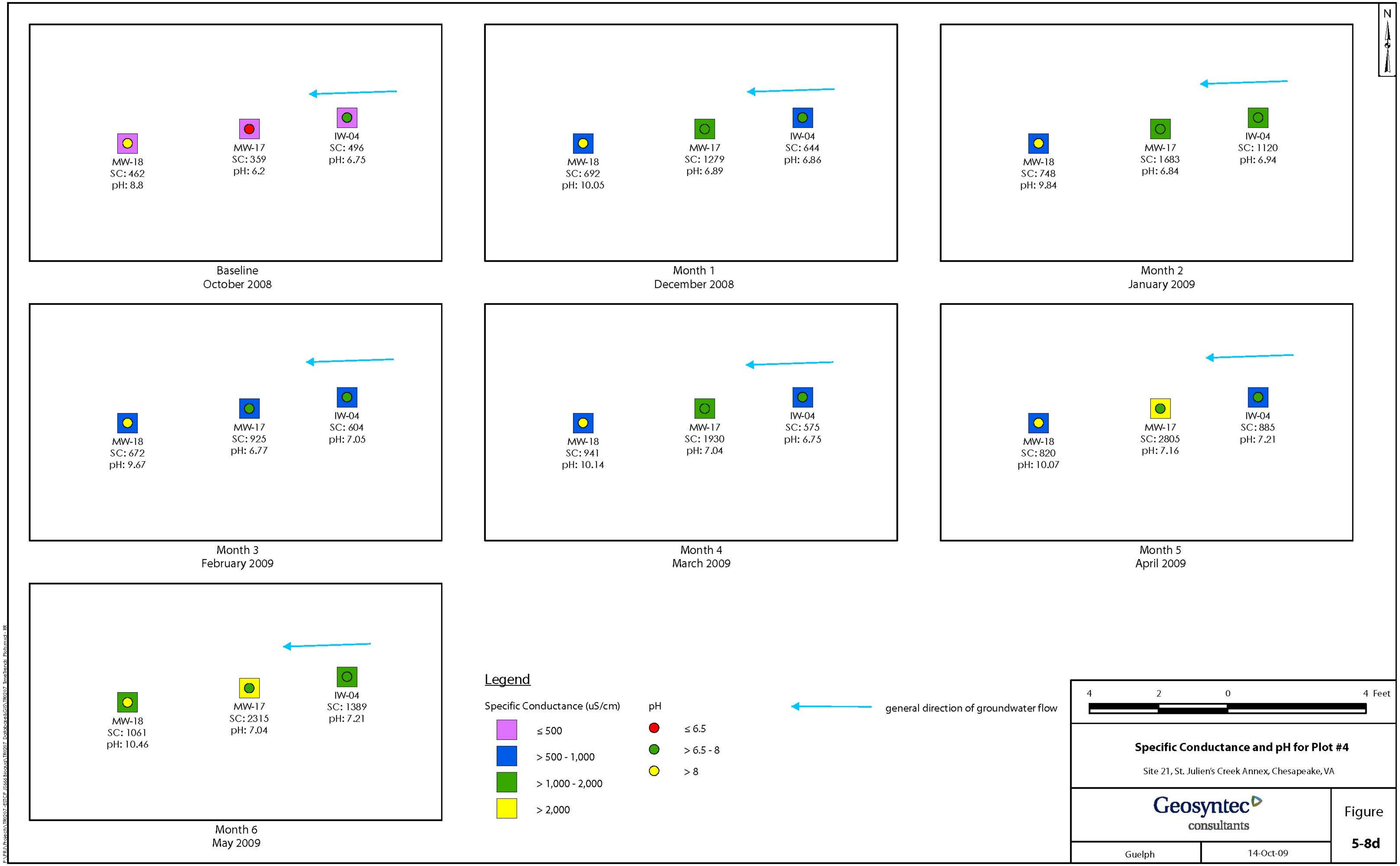
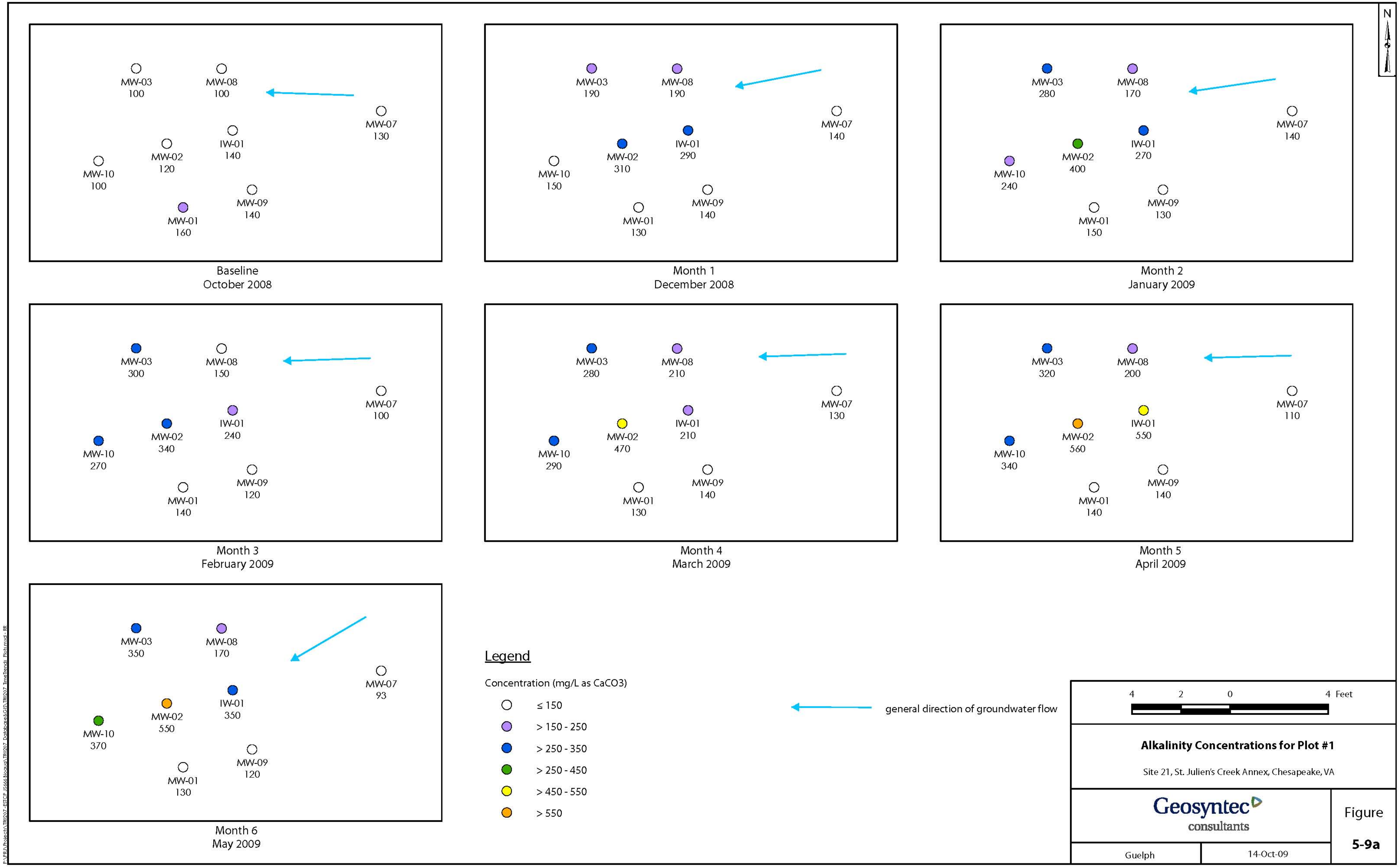
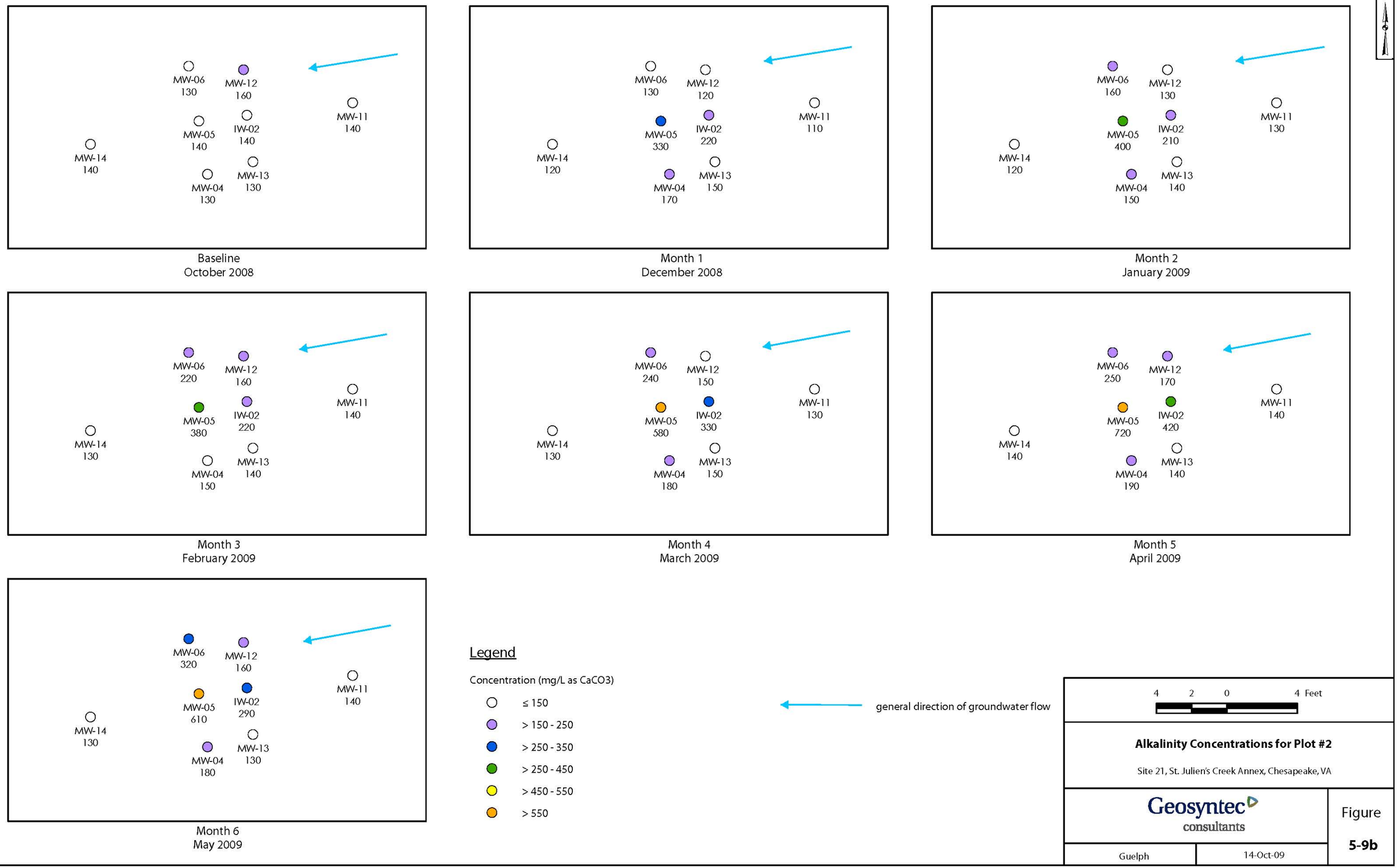
Figure **5-8b**

F:\PEA\Projects\TR0207-ESTCP_IS66 Nicole\TR0207 Outputs\arcfs\GIS\TR0207 TimeTrends Plots.mxd - RR

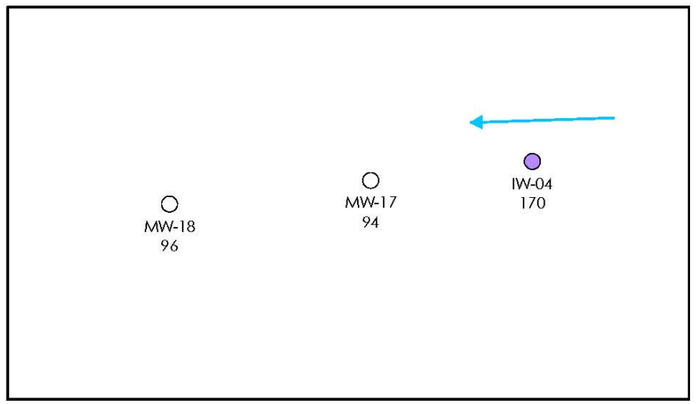

N
MW-16
SC: 361
pH: 6.34
MW-15
SC: 384
pH: 6.39
IW-03
SC: 548
pH: 6.51
Baseline
October 2008
MW-16
SC: 466
pH: 6.57
MW-15
SC: 721
pH: 6.73
IW-03
SC: 1051
pH: 6.89
Month 1
December 2008
MW-16
SC: 855
pH: 6.41
MW-15
SC: 1671
pH: 6.96
IW-03
SC: 1609
pH: 6.96
Month 2
January 2009
MW-16
SC: 1681
pH: 7.18
MW-15
SC: 1298
pH: 7.02
IW-03
SC: 1128
pH: 7.03
Month 3
February 2009
MW-16
SC: 1125
pH: 6.97
MW-15
SC: 2001
pH: 7.13
IW-03
SC: 1989
pH: 7.11
Month 4
March 2009
MW-16
SC: 1001
pH: 7.03
MW-15
SC: 2101
pH: 7.16
IW-03
SC: 1699
pH: 7.12
Month 5
April 2009
MW-16
SC: 740
pH: 6.79
MW-15
SC: 1625
pH: 7.07
IW-03
SC: 1474
pH: 7.04
Month 6
May 2009
Legend
Specific Conductance (uS/cm)
≤ 500
> 500 - 1,000
> 1,000 - 2,000
> 2,000
pH
≤ 6.5
> 6.5 - 8
> 8
general direction of groundwater flow
4 2 0 4 Feet
Specific Conductance and pH for Plot #3
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Guelph
14-Oct-09
Figure
5-8c
F:\PRJ\Projects\TR0207-ESTCP_JS666 Biocaps\TR0207_DataBase\GIS\TR0207_TimeTrends_Plot.mxd - RR


MW-18
SC: 462
pH: 8.8
MW-17
SC: 359
pH: 6.2
IW-04
SC: 496
pH: 6.75
Baseline
October 2008
MW-18
SC: 692
pH: 10.05
MW-17
SC: 1279
pH: 6.89
IW-04
SC: 644
pH: 6.86
Month 1
December 2008
MW-18
SC: 748
pH: 9.84
MW-17
SC: 1683
pH: 6.84
IW-04
SC: 1120
pH: 6.94
Month 2
January 2009
MW-18
SC: 672
pH: 9.67
MW-17
SC: 925
pH: 6.77
IW-04
SC: 604
pH: 7.05
Month 3
February 2009
MW-18
SC: 941
pH: 10.14
MW-17
SC: 1930
pH: 7.04
IW-04
SC: 575
pH: 6.75
Month 4
March 2009
MW-18
SC: 820
pH: 10.07
MW-17
SC: 2805
pH: 7.16
IW-04
SC: 885
pH: 7.21
Month 5
April 2009
MW-18
SC: 1061
pH: 10.46
MW-17
SC: 2315
pH: 7.04
IW-04
SC: 1389
pH: 7.21
Month 6
May 2009
Legend
Specific Conductance (uS/cm)
≤ 500
> 500 - 1,000
> 1,000 - 2,000
> 2,000
pH
≤ 6.5
> 6.5 - 8
> 8
general direction of groundwater flow
4 2 0 4 Feet
N
Specific Conductance and pH for Plot #4
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Figure
5-8d
Guelph
14-Oct-09

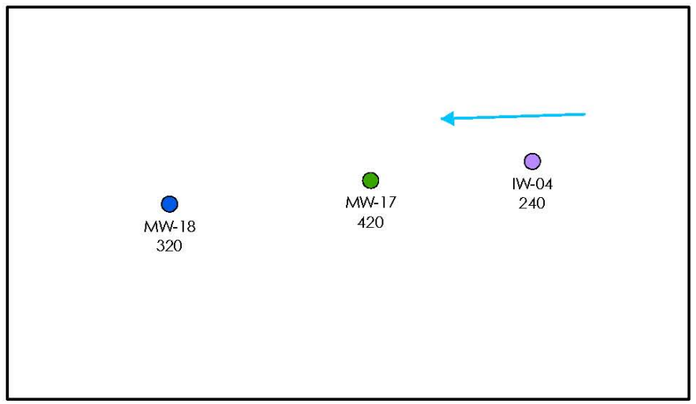

N
MW-03
100
MW-08
100
MW-07
130
MW-02
120
IW-01
140
MW-10
100
MW-09
140
MW-01
160
Baseline
October 2008
MW-03
190
MW-08
190
MW-07
140
MW-02
310
IW-01
290
MW-10
150
MW-09
140
MW-01
130
Month 1
December 2008
MW-03
280
MW-08
170
MW-07
140
MW-02
400
IW-01
270
MW-10
240
MW-09
130
MW-01
150
Month 2
January 2009
MW-03
300
MW-08
150
MW-07
100
MW-02
340
IW-01
240
MW-10
270
MW-09
120
MW-01
140
Month 3
February 2009
MW-03
280
MW-08
210
MW-07
130
MW-02
470
IW-01
210
MW-10
290
MW-09
140
MW-01
130
Month 4
March 2009
MW-03
320
MW-08
200
MW-07
110
MW-02
560
IW-01
550
MW-10
340
MW-09
140
MW-01
140
Month 5
April 2009
MW-03
350
MW-08
170
MW-07
93
MW-02
550
IW-01
350
MW-10
370
MW-09
120
MW-01
130
Month 6
May 2009
Legend
Concentration (mg/L as CaCO3)
≤ 150
> 150 - 250
> 250 - 350
> 250 - 450
> 450 - 550
> 550
general direction of groundwater flow
4 2 0 4 Feet
Alkalinity Concentrations for Plot #1
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec
consultants
Figure
5-9a
Guelph
14-Oct-09


N
MW-06 130
MW-12 160
MW-11 140
MW-05 140
IW-02 140
MW-14 140
MW-13 130
MW-04 130
Baseline
October 2008
MW-06 130
MW-12 120
MW-11 110
MW-05 330
IW-02 220
MW-14 120
MW-13 150
MW-04 170
Month 1
December 2008
MW-06 160
MW-12 130
MW-11 130
MW-05 400
IW-02 210
MW-14 120
MW-13 140
MW-04 150
Month 2
January 2009
MW-06 220
MW-12 160
MW-11 140
MW-05 380
IW-02 220
MW-14 130
MW-13 140
MW-04 150
Month 3
February 2009
MW-06 240
MW-12 150
MW-11 130
MW-05 580
IW-02 330
MW-14 130
MW-13 150
MW-04 180
Month 4
March 2009
MW-06 250
MW-12 170
MW-11 140
MW-05 720
IW-02 420
MW-14 140
MW-13 140
MW-04 190
Month 5
April 2009
MW-06 320
MW-12 160
MW-11 140
MW-05 610
IW-02 290
MW-14 130
MW-13 130
MW-04 180
Month 6
May 2009
Legend
Concentration (mg/L as CaCO3)
≤ 150
> 150 - 250
> 250 - 350
> 250 - 450
> 450 - 550
> 550
general direction of groundwater flow
4 2 0 4 Feet
Alkalinity Concentrations for Plot #2
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Guelph
14-Oct-09
Figure
5-9b

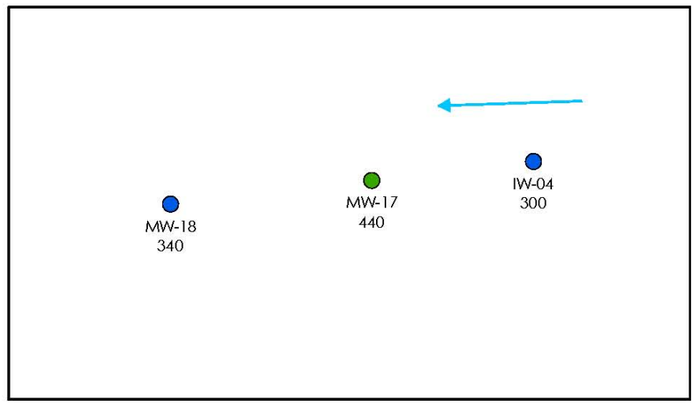

N
MW-16
140
MW-15
140
IW-03
200
Baseline
October 2008
MW-16
150
MW-15
220
IW-03
350
Month 1
December 2008
MW-16
210
MW-15
450
IW-03
490
Month 2
January 2009
MW-16
410
MW-15
410
IW-03
370
Month 3
February 2009
MW-16
330
MW-15
490
IW-03
550
Month 4
March 2009
MW-16
260
MW-15
530
IW-03
520
Month 5
April 2009
MW-16
280
MW-15
510
IW-03
450
Month 6
May 2009
Legend
Concentration (mg/L as CaCO3)
≤ 150
> 150 - 250
> 250 - 350
> 250 - 450
> 450 - 550
> 550
general direction of groundwater flow
4 2 0 4 Feet
Alkalinity Concentrations for Plot #3
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec
consultants
Figure
5-9c
Guelph
14-Oct-09



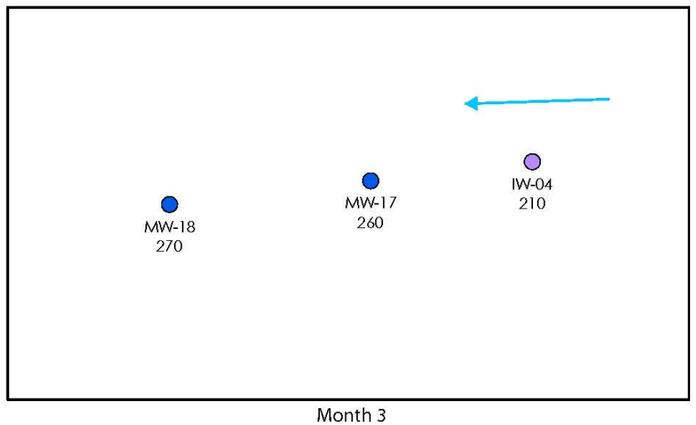
Baseline
October 2008



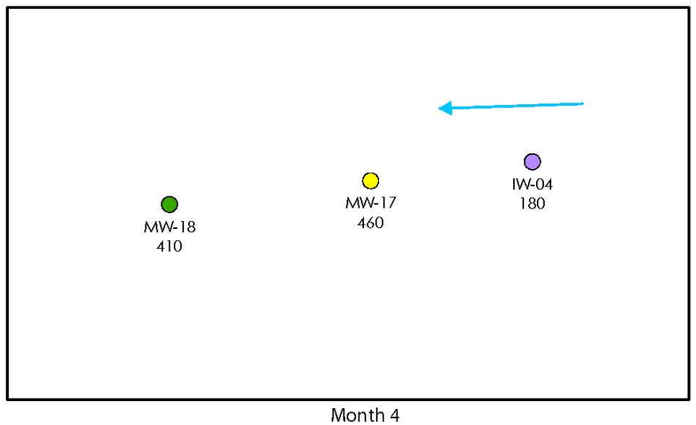
Month 1
December 2008



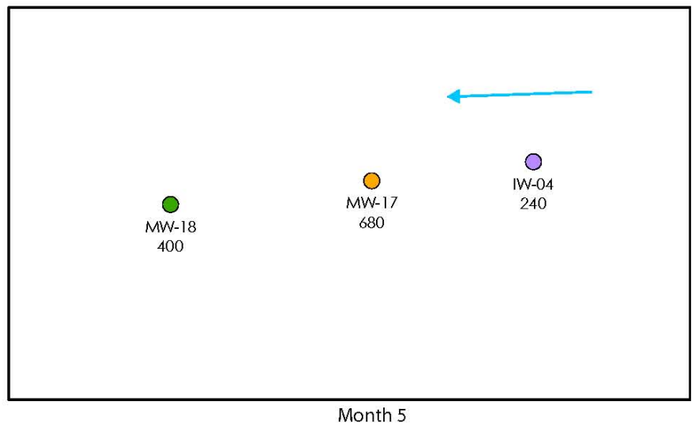
Month 2
January 2009



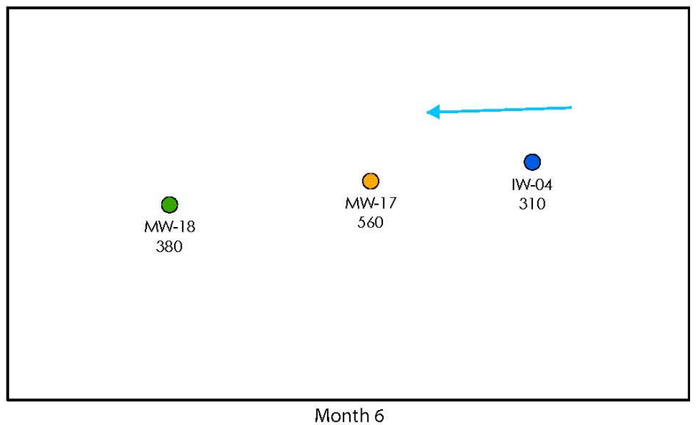
Month 3
February 2009



Month 4
March 2009



Month 5
April 2009



Month 6
May 2009

Legend

Concentration (mg/L as CaCO3)

- ≤ 150
- > 150 - 250
- > 250 - 350
- > 250 - 450
- > 450 - 550
- > 550

general direction of groundwater flow

4 2 0 4 Feet

Alkalinity Concentrations for Plot #4

Site 21, St. Julien's Creek Annex, Chesapeake, VA

Geosyntec consultants

Guelph | 14-Oct-09

Figure **5-9d**

P:\PFA Projects\TR0707 - ESTCP #5566 Norcom\TR0707 Database and GIS\TR0707 TimeTrends Plots.mxd - RR

transgradient wells. Like the trends observed with the specific conductance and pH, minimal increases in alkalinity were observed in MW-09, MW-01, and MW-13. No significant change in alkalinity was observed in the upgradient wells for either bioaugmentation plot, indicating that downgradient increases were attributed to microbial activity stimulated by buffer addition and/or JS666 bioaugmentation.

Concentrations of dissolved manganese in the four injection wells and downgradient monitoring wells MW-02, MW-03, MW-10, MW-15, MW-16 and MW-17 decreased over the study duration, most likely due to increasing pH levels in these wells, and thus formation of manganese hydroxides. Dissolved manganese concentrations in other monitoring wells, including the upgradient monitoring wells MW-07 and MW-11, varied slightly but ultimately returned to near baseline concentrations during the final sampling event. Concentrations of dissolved iron in almost all monitoring wells were more variable than dissolved manganese. However, the four injection wells all showed reductions in dissolved iron over the project duration, likely as a result of addition of air or aerated buffer.

Methane concentrations in Plots 1, 2 and 3 varied for most wells, with levels ranging from 43 to 940 µg/L, but the levels were generally not indicative of deeply reduced conditions. The exceptions to this observation were the methane concentrations in all wells in Plot #4, which increased over the project duration. Methane concentrations in the two downgradient monitoring wells, MW-17 and MW-18, increased from 960 µg/L to 2,800 µg/L and from 2,200 µg/L to 12,000 µg/L, respectively. The reason for this is not clear.

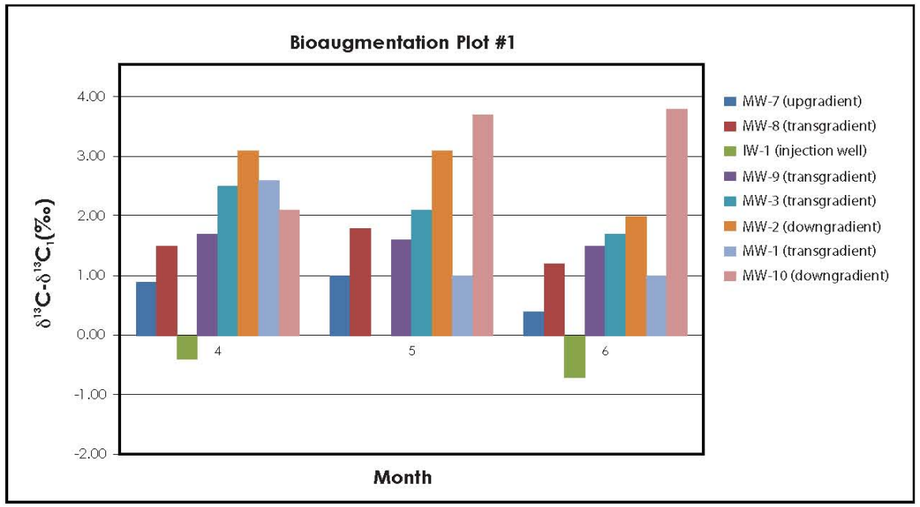
5.7.4 Isotopic Analyses

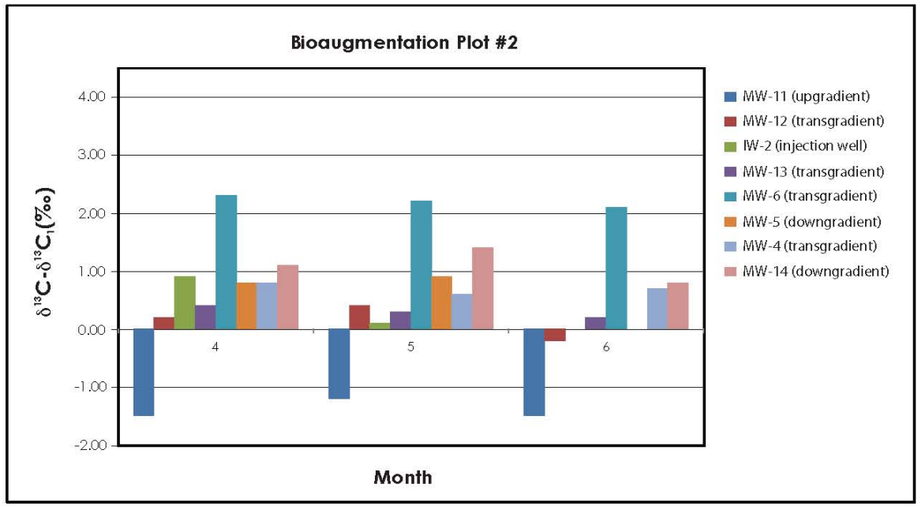
Results of cDCE isotope analyses are presented in Figures F-1a through F-4c in Appendix F. In addition, bar charts showing changes in $\delta^{13}C$ in cDCE compared to the month 1 sampling event can be found in Figure 5-10. Trends observed in the control and bioaugmentation plots are presented below.

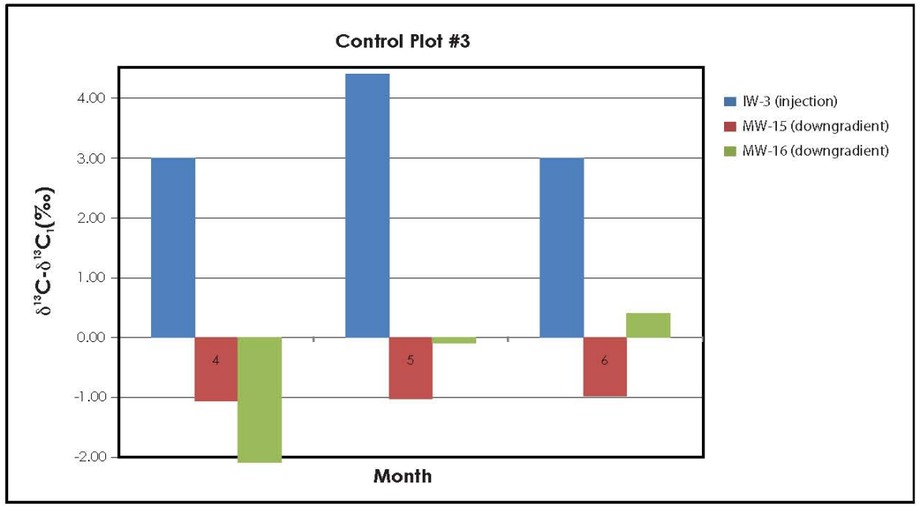
Control Plots

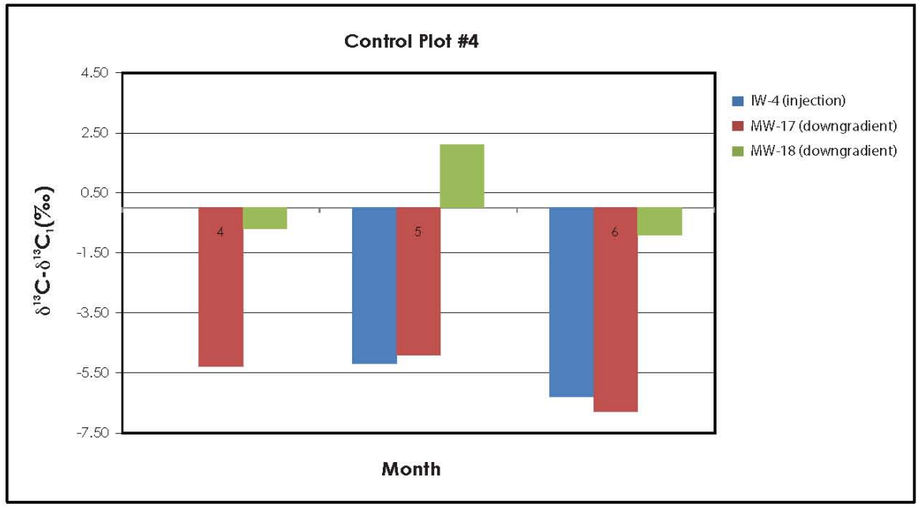
All monitoring wells in both Control Plots #3 and #4 showed substantial isotopic enrichment between the first two sampling dates, consistent with significant biodegradation of cDCE in those areas of the plume (Figures F-3a to F-4c, in Appendix F). Thereafter however, while concentration levels increase and decrease over time in these wells, $\delta^{13}C$ values for cDCE showed little or only a small degree of continued enrichment (IW-3, MW-15, MW-16, MW-18) or there was a reversal of the enrichment trend, and $\delta^{13}C$ values became less enriched (IW-4, MW-17).

Figure 5-10 presents the data in a different way by showing the change in $\delta^{13}C$ relative to the $\delta^{13}C$ levels during the Month 1 sampling event for all wells in Control Plots #3 and #4.









Note:
Error in $\delta^{13}C$ values is +/- 0.5 permil.

DRAFT

Changes in $\delta^{13}C$ in cDCE in Groundwater
Site 21, St. Julien's Creek Annex
Chesapeake, Virginia

Geosyntec consultants

Guelph | January 2010

Figure **5-10**

Changes in d13C.ai

Between Month 4 and Month 6, MW-15, IW4, and MW-17 became less enriched, while IW-3 and MW-18 showed enrichment.

Taken together these results indicate that the main control on cDCE concentrations in the control plots was not biodegradation but fluctuations due to pumping and/or groundwater transport processes. The possible exception is MW-18 where the changes in VOC concentrations and isotope signatures track quite closely (Figure F-4c) and suggest biodegradation may be occurring in this control well to a greater extent than any of the others. This conclusion is supported as well by the fact that MW-18 shows the most enriched $\delta^{13}C$ value (-15 .2 permil) in any of the control wells on the second to last sampling date. Higher VC levels and a lower ORP in this well suggest reductive dechlorination was occurring rather than degradation attributable to JS666.

Bioaugmentation Plots #1 and #2

All wells in Bioaugmentaton Plot #1 showed trends of isotopic enrichment over the study consistent with the effects of biodegradation (Figures F-1b to F-1h, Appendix F). The most consistent trends and most pronounced isotopic enrichments (up to 4-5 permil) were observed in downgradient wells MW-2, MW-3, and MW-10. A test of the fit of a Rayleigh model to these 3 wells showed correlation coefficients (r^2) of 0.8, 0.6 and 0.6 respectively. This is not an exceptional fit to the model; nonetheless these fits are not unreasonable for field data and hence an apparent fractionation factor can be calculated for comparative purposes. These calculations showed apparent ε values of –7.7, -7.9 and –8.1, respectively. Although there was some enrichment in upgradient well MW-07, it was generally less than 1 permil. Recall that the uncertainty in the measurement is +/- 0.5 permil.

With the exception of the upgradient well MW-11, all wells in Bioaugmentation Plot #2 showed trends of isotopic enrichment over the study consistent with the effects of biodegradation. Four wells had correlation coefficients (r^2) in the range of 0.6 to 0.9 for a test of the Rayleigh model and corresponding apparent fractionation factors or ε values from –3.7 to –9.0 using data from October 2008 to April 2009. Well MW-11 showed substantial isotopic enrichment between the first two sampling dates, but thereafter showed a general reversal of the enrichment trend, with $\delta^{13}C$ values becoming less enriched.

Figure 5-10 shows changes in $\delta^{13}C$ relative to Month 1 values (i.e., $\delta^{13}C_t - \delta^{13}C_1$) for Months 4 to 6. The monitoring wells in Bioaugmentation Plot #1 show substantial enrichment, while the monitoring wells in the corresponding Control Plot #4 do not (with the exception of MW-18). These results suggest that biodegradation was occurring primarily because of the addition of JS666 rather than the addition of buffer. Figure 5-10 also indicates that there was modestly more overall enrichment in Bioaugmentaiton Plot #2 relative to Control Plot #3, suggesting a modest effect of JS666 relative to buffer alone. Plots #2 and #3 did not receive air via the Waterloo emitter and, therefore, may have been oxygen-limited. In conclusion, the CSIA results support a significant degree of biodegradation in downgradient wells in Bioaugmentation Plots #1 and #2

and more biodegradation in bioaugmentation plots relative to control plots that received buffer but not JS666.

Apparent fractionation factors for field data are typically somewhat smaller than lab-derived factors because, in the field, changes in concentrations are affected by physical processes of contaminant mass reduction, while carbon isotope values for chlorinated ethenes are typically not (Hunkeler *et al*., 2008). Hence a finding that apparent fractionation factors calculated from field data are smaller than lab-derived ones is not unexpected. Interestingly, 4 wells overall (MW-12, MW-10, MW-2 and MW-3) have apparent fractionation factors in the range of –7 to –9 – quite close to the published fractionation factor for aerobic cDCE biodegradation by JS666 (Abe et al., 2009). Overall, while this does not rule out the possibility that a different native organism using the same pathway as JS666 is active at the site, it is nonetheless also consistent with the hypothesis that the observed biodegradation of cDCE is due to JS666.

5.7.5 Volatile Organic Compound Data

TCE

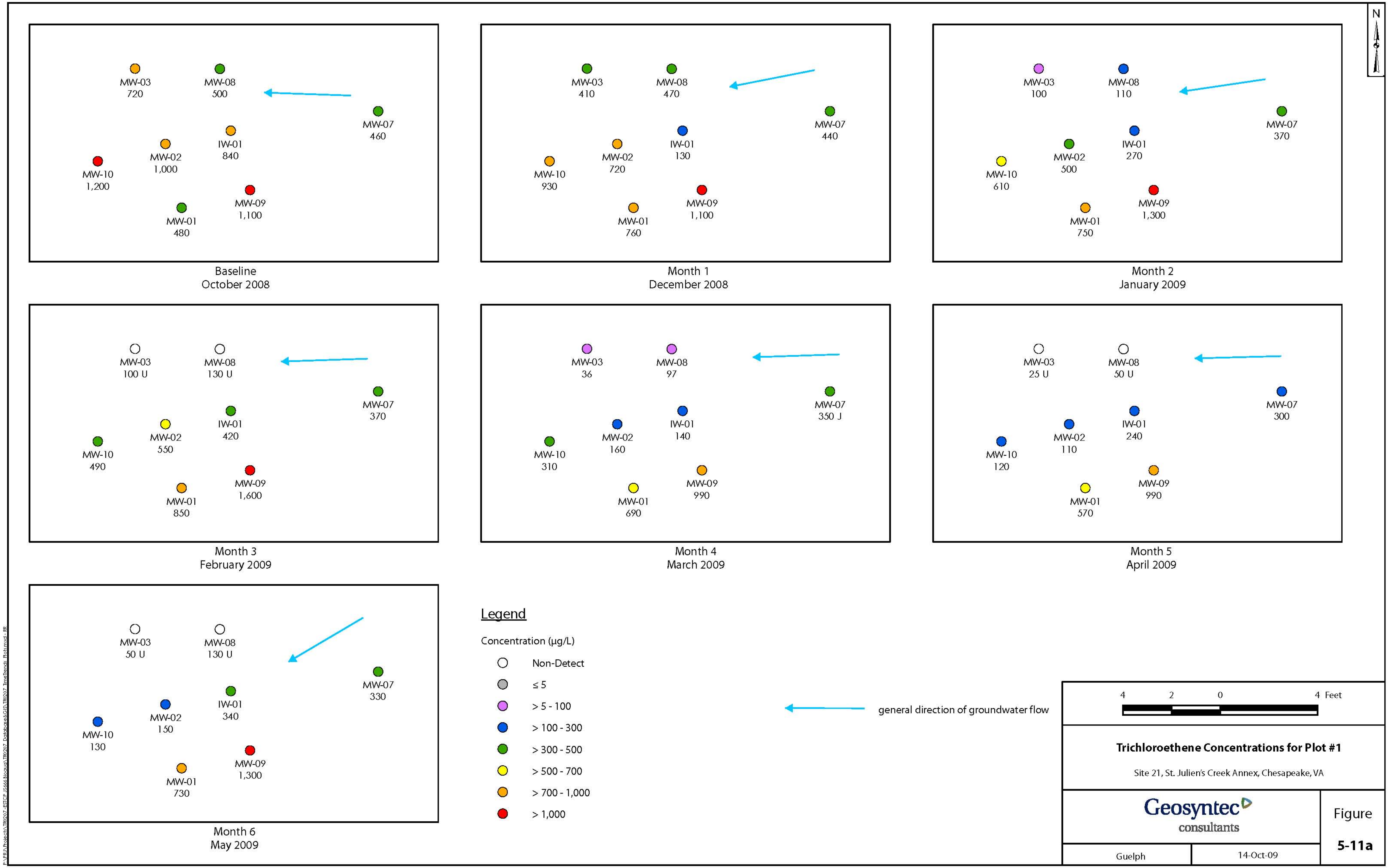
Almost all wells in Bioaugmentation Plot #1 (with the exception of MW-01 and MW-09) exhibited considerable reductions in TCE over the course of the demonstration (Figure 5-11a). TCE concentrations in the upgradient well, MW-07, remained relatively constant throughout the demonstration. Given the high rates of TCE removal in the control plots (discussed below), the TCE reduction in this plot is likely due to biodegradation by bacteria other than JS666.

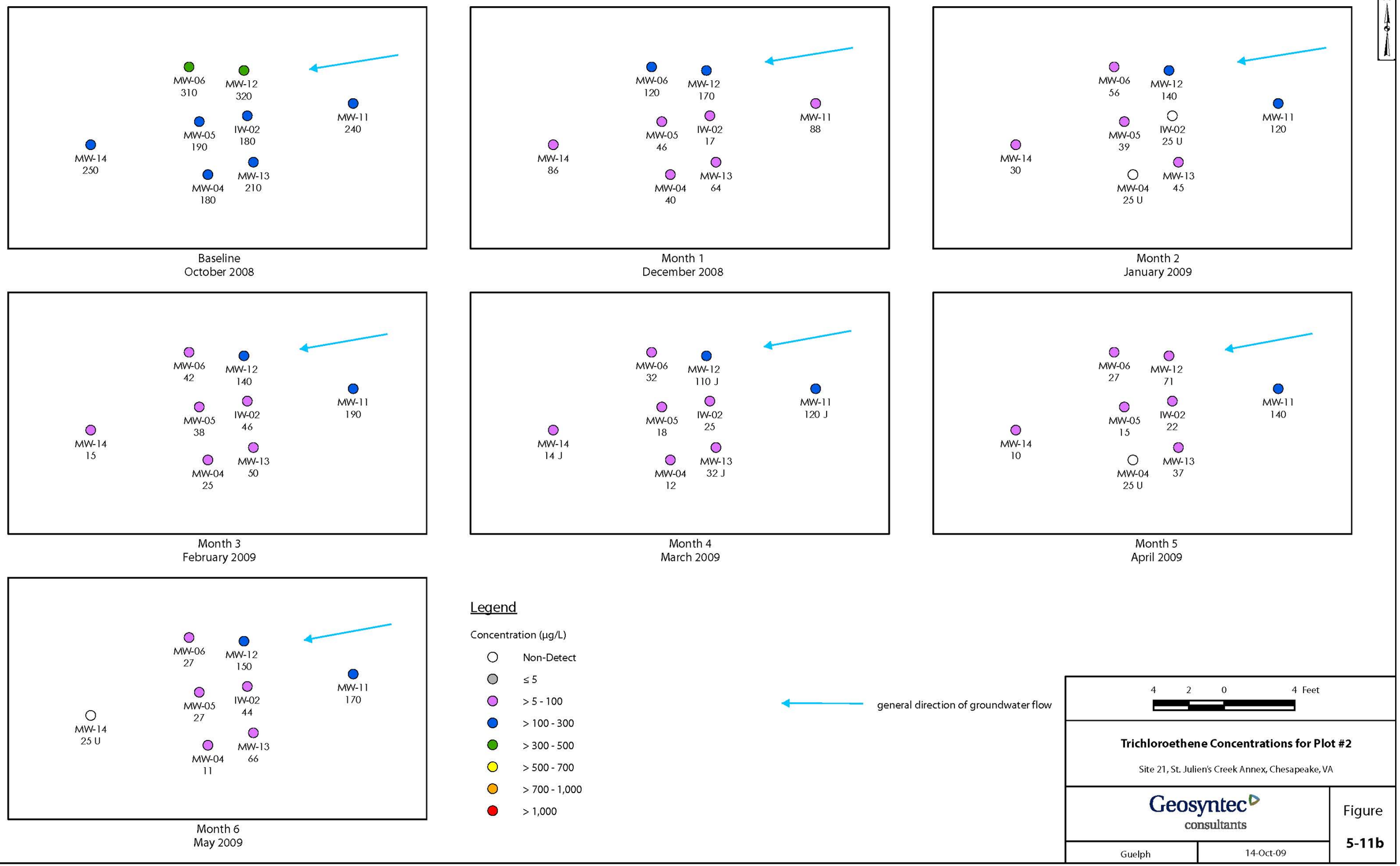
All downgradient wells in Bioaugmentation Plot #2 exhibited considerable reductions in TCE following the first bioaugmentation, and levels remained low throughout the remainder of the demonstration (Figure 5-11b). TCE concentration in the upgradient well, MW-11, fluctuated but were generally considerably higher than in downgradient wells. Given the high rates of TCE removal in the Control Plot #3 (discussed below), the TCE reduction in this bioaugmentation plot is likely due to biodegradation by bacteria other than JS666

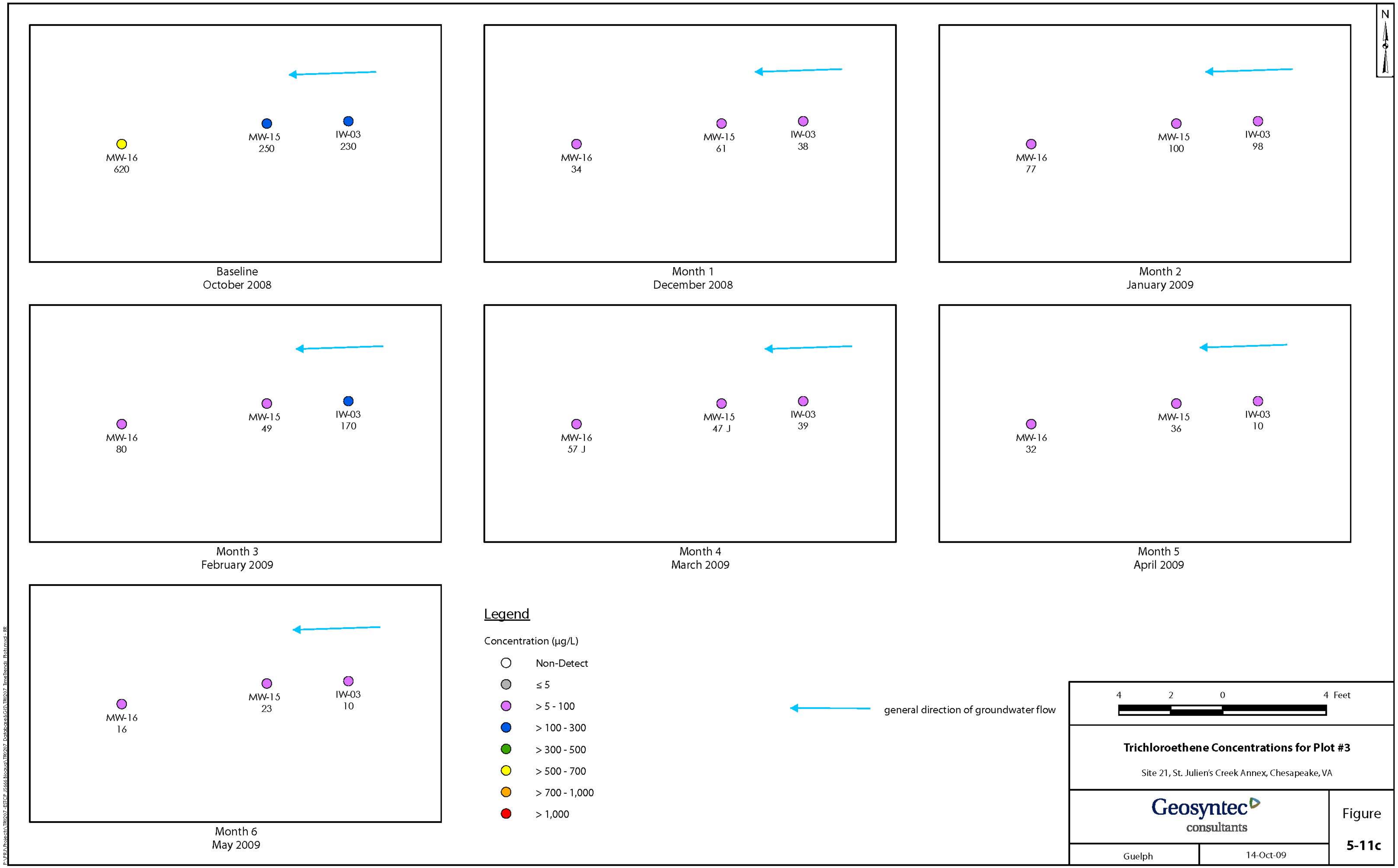
All wells in Plot #3 and Plot #4 exhibited considerable and sustained reductions in TCE over the course of the demonstration (Figures 5-11c and 5-11d), suggesting the addition of buffer alone had stimulated TCE biodegradation.

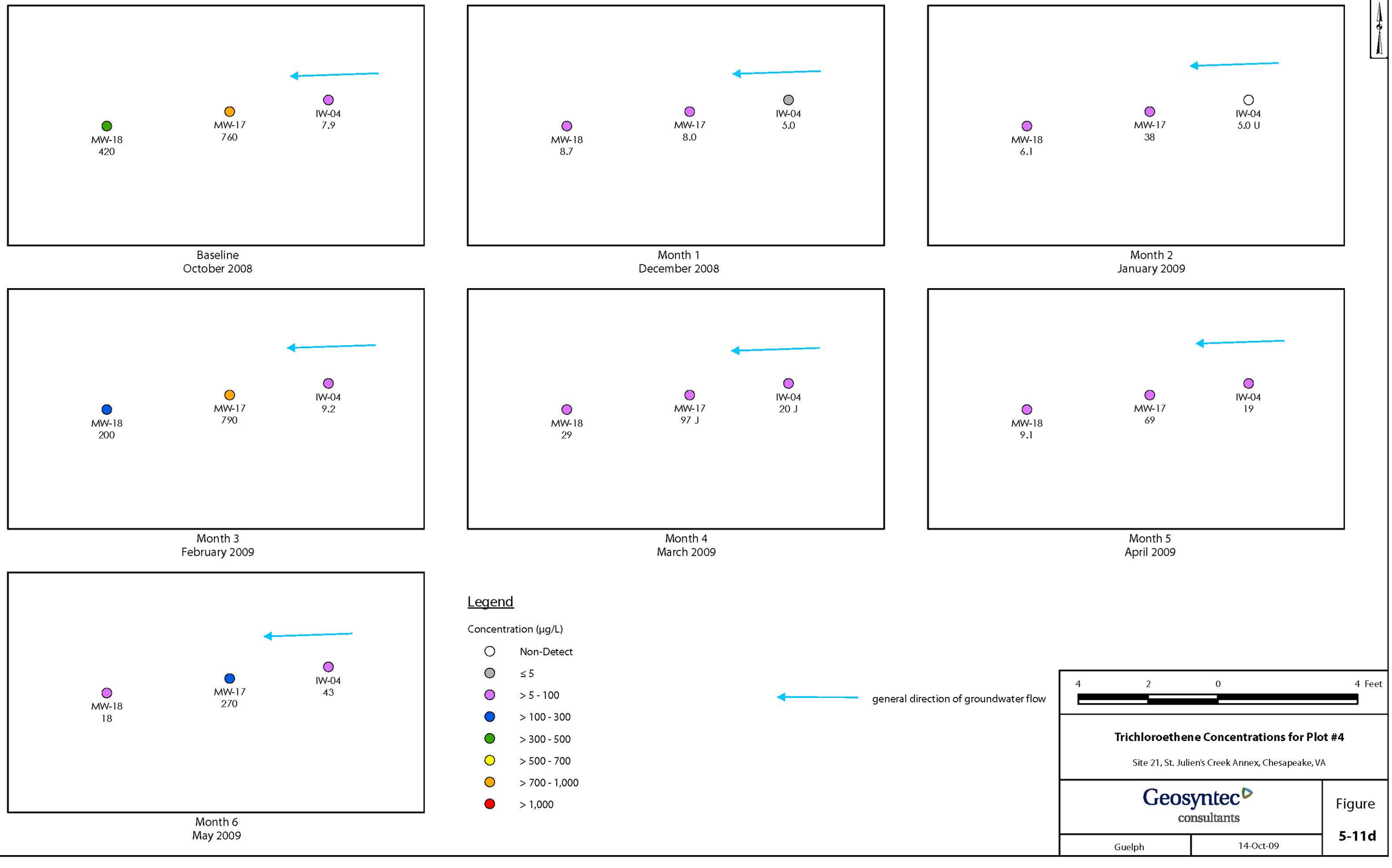
cDCE

In Bioaugmentation Plot #1, cDCE concentrations in the upgradient unamended well MW-7 increased after October 2008, with an average cDCE concentration of 2533 ug/L from October 2008 to April 2009. Average cDCE concentrations in downgradient wells decreased 7-44% relative to upgradient cDCE concentrations. (Note that May 2009 data were excluded due the malfunctioning of the air cylinder supplying the Waterloo Emitters.) Of the 8 wells in


N
MW-03
720
MW-08
500
MW-07
460
MW-02
1,000
IW-01
840
MW-10
1,200
MW-09
1,100
MW-01
480
Baseline
October 2008
MW-03
410
MW-08
470
MW-07
440
MW-02
720
IW-01
130
MW-10
930
MW-09
1,100
MW-01
760
Month 1
December 2008
MW-03
100
MW-08
110
MW-07
370
MW-02
500
IW-01
270
MW-10
610
MW-09
1,300
MW-01
750
Month 2
January 2009
MW-03
100 U
MW-08
130 U
MW-07
370
MW-02
550
IW-01
420
MW-10
490
MW-09
1,600
MW-01
850
Month 3
February 2009
MW-03
36
MW-08
97
MW-07
350 J
MW-02
160
IW-01
140
MW-10
310
MW-09
990
MW-01
690
Month 4
March 2009
MW-03
25 U
MW-08
50 U
MW-07
300
MW-02
110
IW-01
240
MW-10
120
MW-09
990
MW-01
570
Month 5
April 2009
MW-03
50 U
MW-08
130 U
MW-07
330
MW-02
150
IW-01
340
MW-10
130
MW-09
1,300
MW-01
730
Month 6
May 2009
Legend
Concentration (µg/L)
Non-Detect
≤ 5
> 5 - 100
> 100 - 300
> 300 - 500
> 500 - 700
> 700 - 1,000
> 1,000
general direction of groundwater flow
4 2 0 4 Feet
Trichloroethene Concentrations for Plot #1
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Figure
5-11a
Guelph
14-Oct-09


N
MW-06 310
MW-12 320
MW-11 240
MW-05 190
IW-02 180
MW-14 250
MW-13 210
MW-04 180
Baseline October 2008
MW-06 120
MW-12 170
MW-11 88
MW-05 46
IW-02 17
MW-14 86
MW-13 64
MW-04 40
Month 1 December 2008
MW-06 56
MW-12 140
MW-11 120
MW-05 39
IW-02 25 U
MW-14 30
MW-13 45
MW-04 25 U
Month 2 January 2009
MW-06 42
MW-12 140
MW-11 190
MW-05 38
IW-02 46
MW-14 15
MW-13 50
MW-04 25
Month 3 February 2009
MW-06 32
MW-12 110 J
MW-11 120 J
MW-05 18
IW-02 25
MW-14 14 J
MW-13 32 J
MW-04 12
Month 4 March 2009
MW-06 27
MW-12 71
MW-11 140
MW-05 15
IW-02 22
MW-14 10
MW-13 37
MW-04 25 U
Month 5 April 2009
MW-06 27
MW-12 150
MW-11 170
MW-05 27
IW-02 44
MW-14 25 U
MW-13 66
MW-04 11
Month 6 May 2009
Legend
Concentration (µg/L)
Non-Detect
≤ 5
> 5 - 100
> 100 - 300
> 300 - 500
> 500 - 700
> 700 - 1,000
> 1,000
general direction of groundwater flow
4 2 0 4 Feet
Trichloroethene Concentrations for Plot #2
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Guelph
14-Oct-09
Figure 5-11b


N
MW-16
620
MW-15
250
IW-03
230
Baseline
October 2008
MW-16
34
MW-15
61
IW-03
38
Month 1
December 2008
MW-16
77
MW-15
100
IW-03
98
Month 2
January 2009
MW-16
80
MW-15
49
IW-03
170
Month 3
February 2009
MW-16
57 J
MW-15
47 J
IW-03
39
Month 4
March 2009
MW-16
32
MW-15
36
IW-03
10
Month 5
April 2009
MW-16
16
MW-15
23
IW-03
10
Month 6
May 2009
Legend
Concentration (µg/L)
Non-Detect
≤ 5
> 5 - 100
> 100 - 300
> 300 - 500
> 500 - 700
> 700 - 1,000
> 1,000
general direction of groundwater flow
4 2 0 4 Feet
Trichloroethene Concentrations for Plot #3
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Figure
5-11c
Guelph
14-Oct-09


N
MW-18
420
MW-17
760
IW-04
7.9
Baseline
October 2008
MW-18
8.7
MW-17
8.0
IW-04
5.0
Month 1
December 2008
MW-18
6.1
MW-17
38
IW-04
5.0 U
Month 2
January 2009
MW-18
200
MW-17
790
IW-04
9.2
Month 3
February 2009
MW-18
29
MW-17
97 J
IW-04
20 J
Month 4
March 2009
MW-18
9.1
MW-17
69
IW-04
19
Month 5
April 2009
MW-18
18
MW-17
270
IW-04
43
Month 6
May 2009
Legend
Concentration (µg/L)
Non-Detect
≤ 5
> 5 - 100
> 100 - 300
> 300 - 500
> 500 - 700
> 700 - 1,000
> 1,000
general direction of groundwater flow
4 2 0 4 Feet
Trichloroethene Concentrations for Plot #4
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Guelph
14-Oct-09
Figure
5-11d

Bioaugmentation Plot #1, IW-01, MW-01, MW-02, and MW-03 showed the greatest degree of cDCE removal (Figure 5-12a).

In Bioaugmentation Plot #2, cDCE concentrations in downgradient wells generally decreased 14-25% relative to average cDCE concentrations in upgradient well MW-11(Figure 5-12b). The exceptions to this trend were MW-4 and MW-13 where average cDCE concentrations increased relative to those in MW-11.

By contrast, in Control Plot #3, cDCE concentrations remained relatively the same throughout the demonstration (Figure 5-12c). In Control Plot #4 (which received an emitter and buffer), wells MW-17 and MW-18 showed initial reductions in cDCE up until the February sampling event, when cDCE rebounded to near baseline conditions (Figure 5-12d). However cDCE concentrations did decrease again immediately following the second bioaugmentation when aeration of the buffer-amended groundwater was initiated.

VC

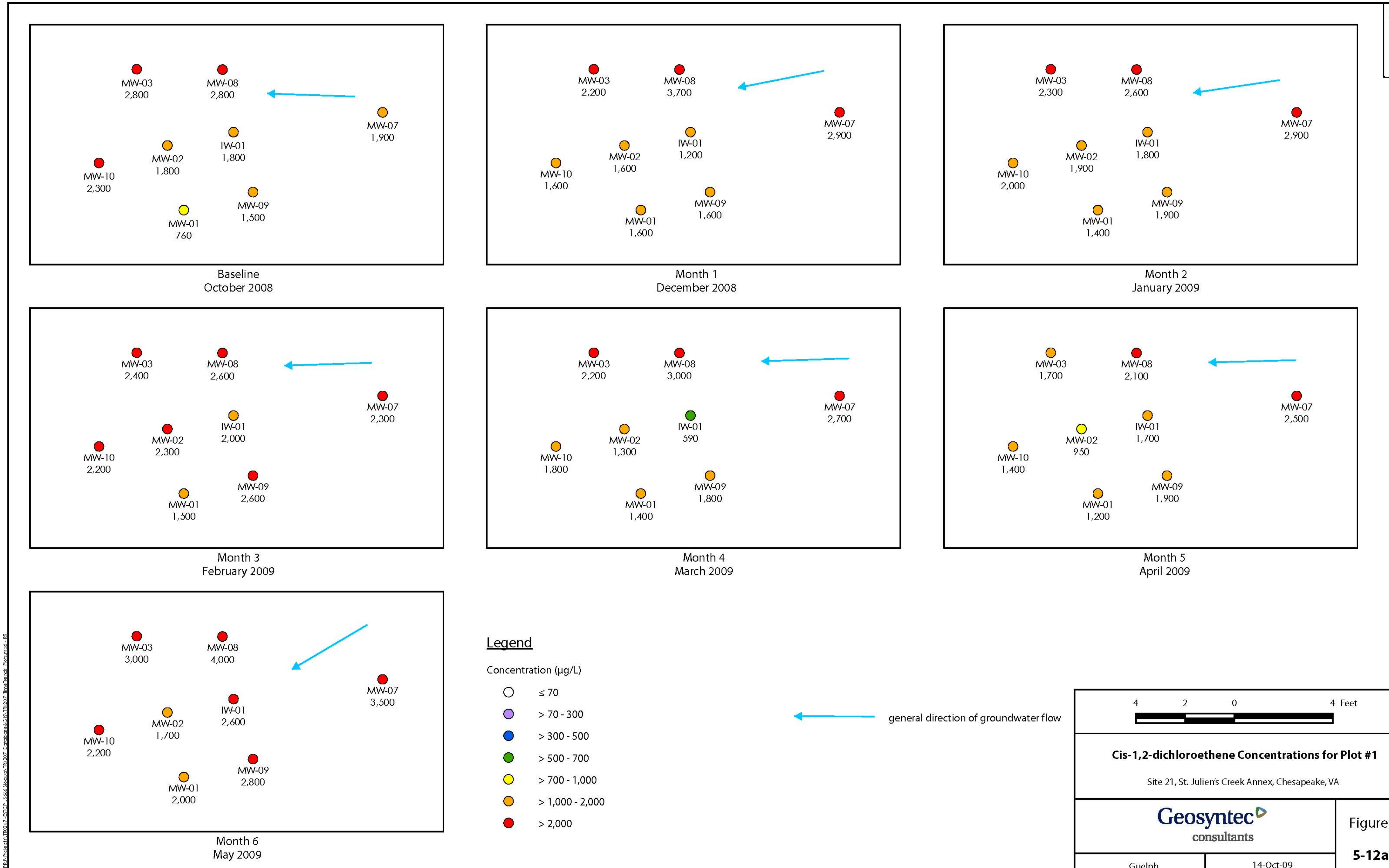
VC concentrations fluctuated over the demonstration period and were generally low in Plots #1 to #3 (Table E-2 in Appendix E), but were highest in MW-18 in Plot #4 (Figure 5-13). MW-18 also had more reduced conditions and a higher pH relative to the other wells. Thus, some of the TCE and cDCE declines in Plot #4 may have been due to reductive dechlorination rather than aerobic processes.

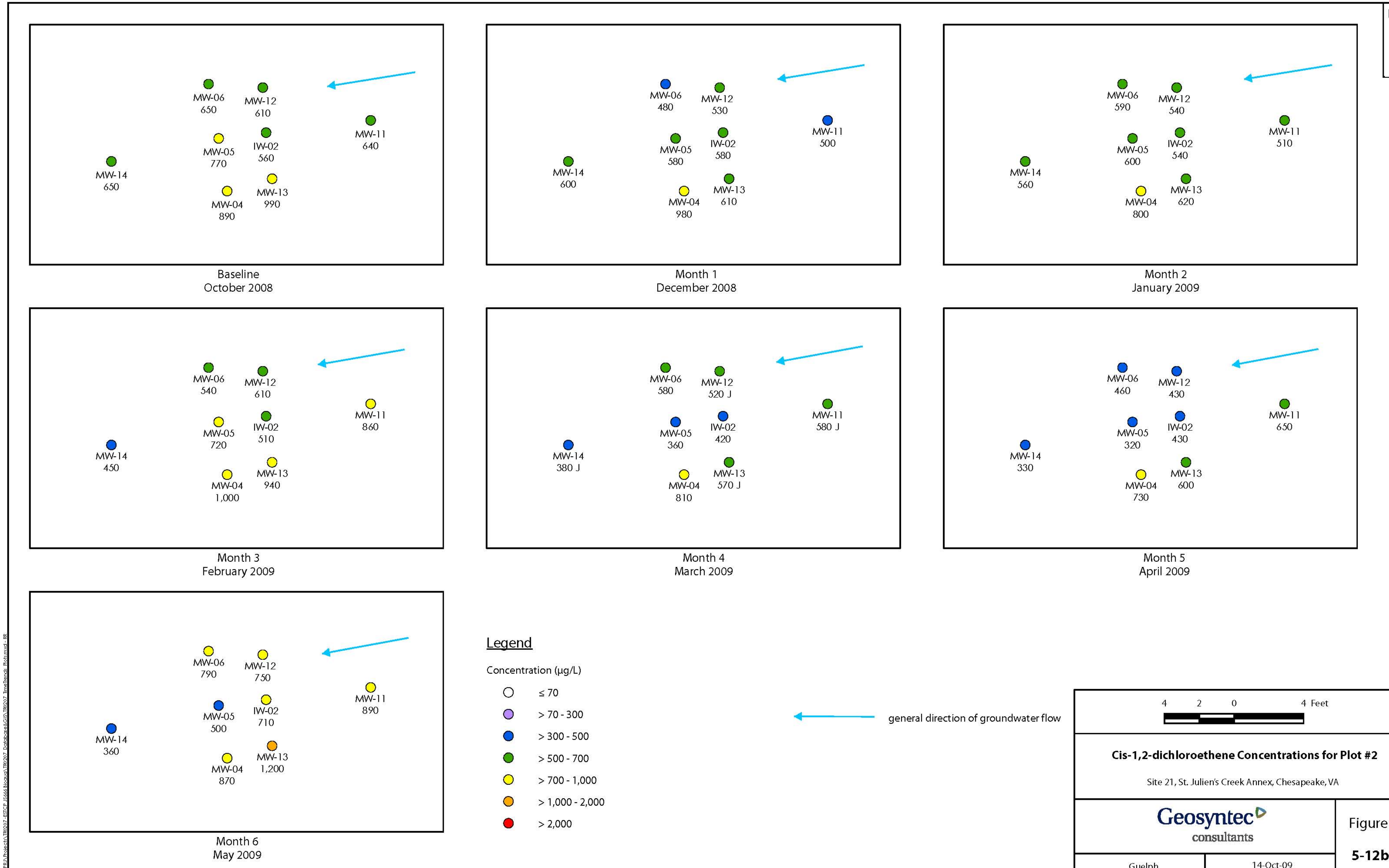
5.7.6 Probe Assay and Microcosm Assay Results

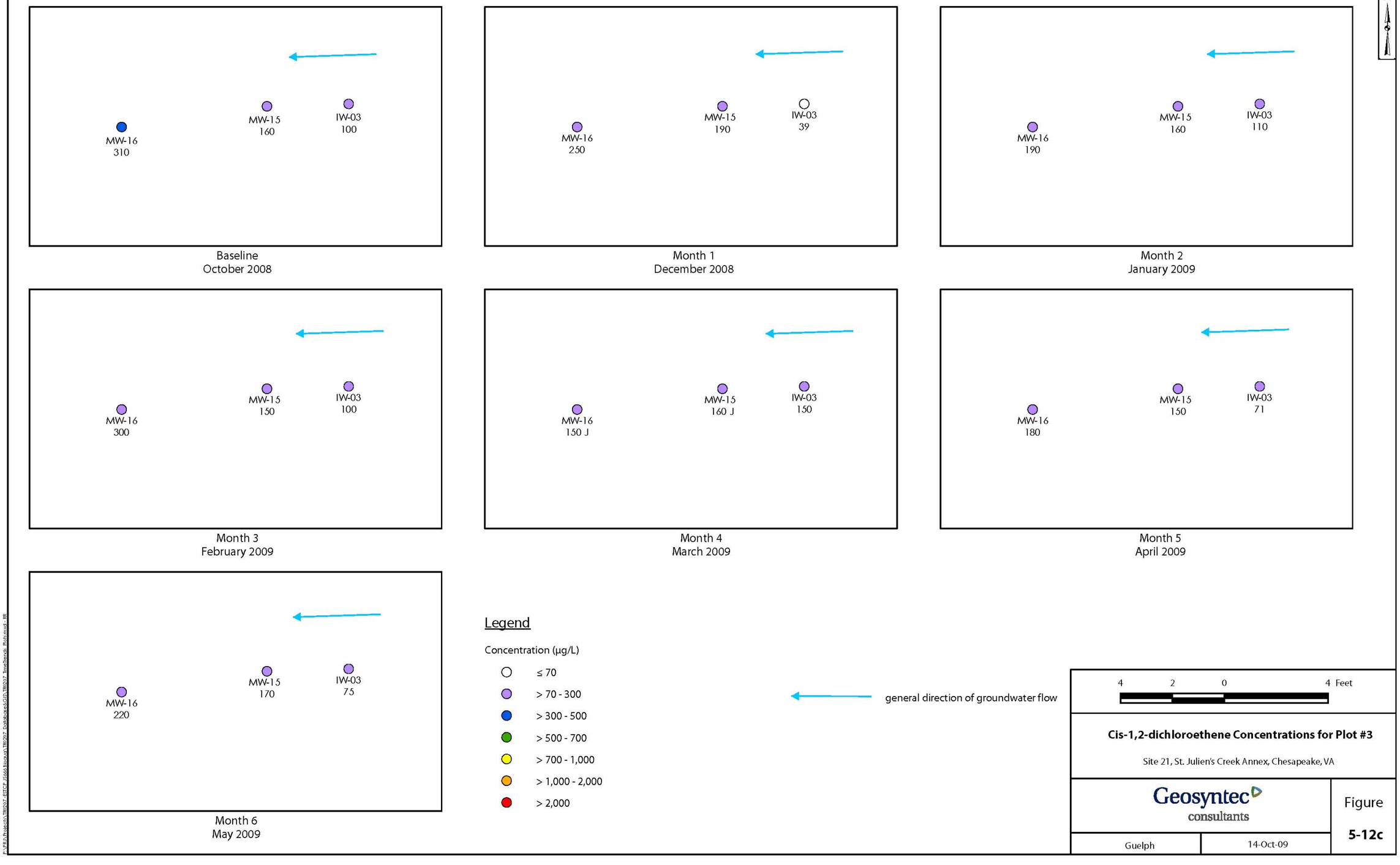
Probe Assay- Inoculum Levels

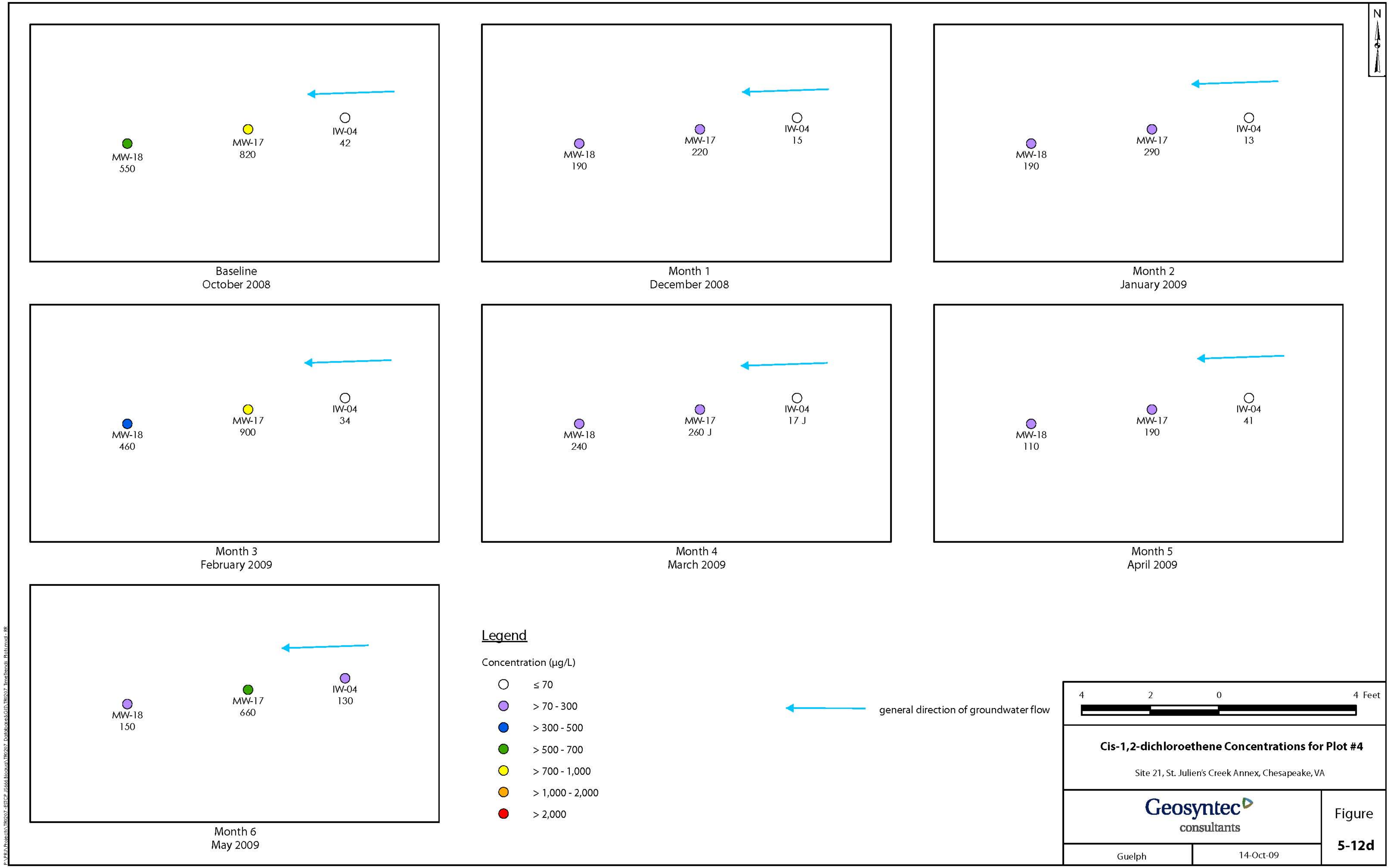
qPCR results for samples of the inoculum culture before injection and from IW-1 and IW-2 after injection for the first bioaugmentation event are shown in Figure 5-14. Since JS666 has only single copies of both the isocitrate lyase gene and the 16S rRNA gene, one would expect comparable results between ISO and UNI probes if the inoculum were pure. [Generally, with pure cultures of JS666, we see ratios of UNI/ISO between 0.5 and 5.] Given that the UNI/ISO ratio in this inoculum was a bit higher, some level of contamination is suggested. Still, the results indicate a JS666 inoculum density of approximately 10^8 per mL prior to injection.

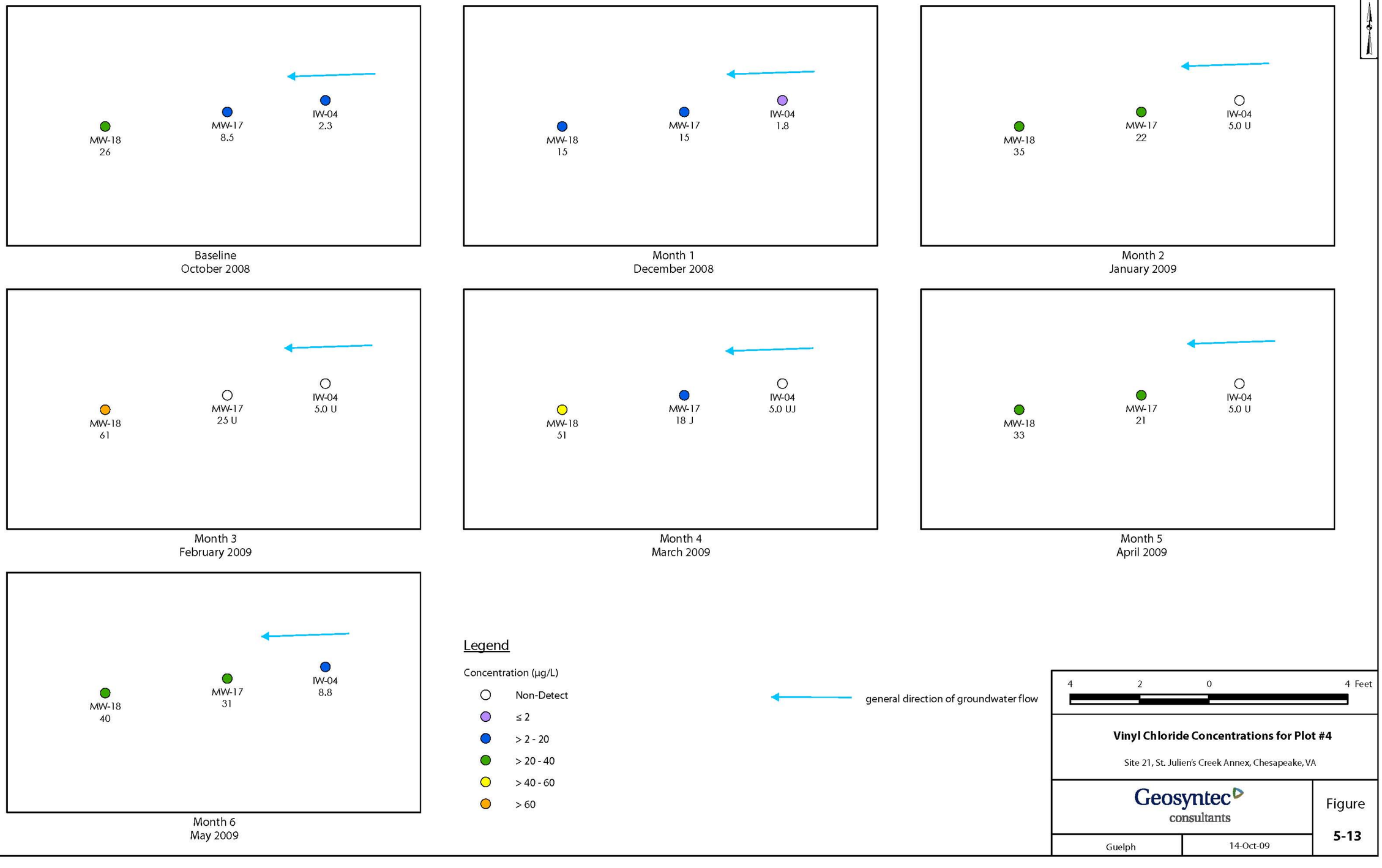
qPCR results for inoculum culture used in the second bioaugmentation event are shown in Figure 5-14. “SJCA” represents a sample of the inoculum sent to Cornell from the field. “GT” represents a sample sent to Cornell directly from Georgia Tech, prior to shipping the inoculum to the field. Again, the results indicate an inoculum density at the field site of approximately 10^8 per mL.

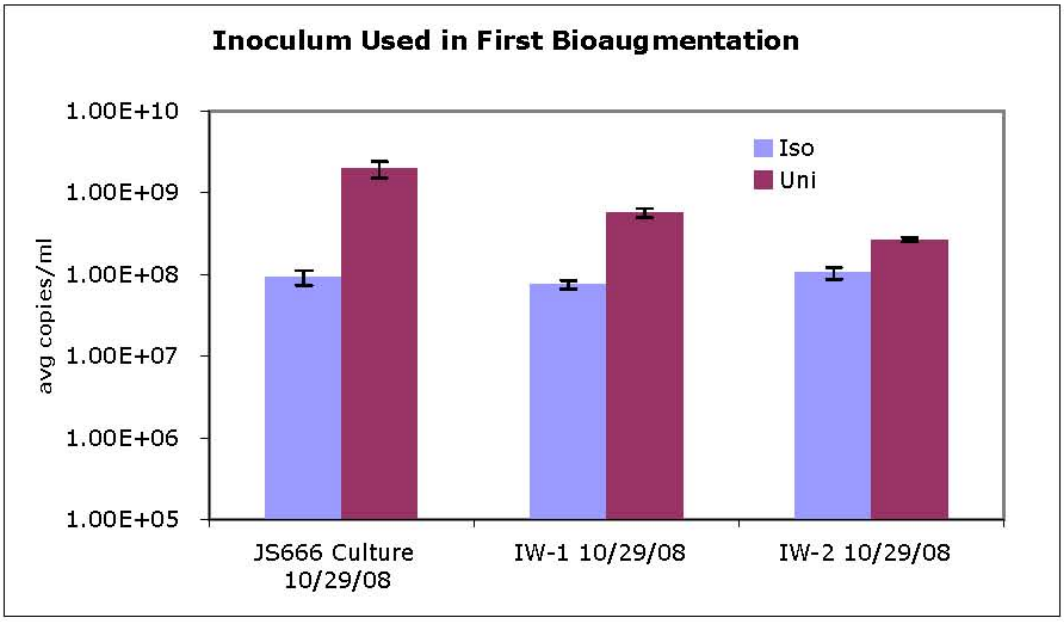

N
MW-03
2,800
MW-08
2,800
MW-07
1,900
MW-10
2,300
MW-02
1,800
IW-01
1,800
MW-09
1,500
MW-01
760
Baseline
October 2008
MW-03
2,200
MW-08
3,700
MW-07
2,900
MW-10
1,600
MW-02
1,600
IW-01
1,200
MW-09
1,600
MW-01
1,600
Month 1
December 2008
MW-03
2,300
MW-08
2,600
MW-07
2,900
MW-10
2,000
MW-02
1,900
IW-01
1,800
MW-09
1,900
MW-01
1,400
Month 2
January 2009
MW-03
2,400
MW-08
2,600
MW-07
2,300
MW-10
2,200
MW-02
2,300
IW-01
2,000
MW-09
2,600
MW-01
1,500
Month 3
February 2009
MW-03
2,200
MW-08
3,000
MW-07
2,700
MW-10
1,800
MW-02
1,300
IW-01
590
MW-09
1,800
MW-01
1,400
Month 4
March 2009
MW-03
1,700
MW-08
2,100
MW-07
2,500
MW-10
1,400
MW-02
950
IW-01
1,700
MW-09
1,900
MW-01
1,200
Month 5
April 2009
MW-03
3,000
MW-08
4,000
MW-07
3,500
MW-10
2,200
MW-02
1,700
IW-01
2,600
MW-09
2,800
MW-01
2,000
Month 6
May 2009
Legend
Concentration (µg/L)
≤ 70
> 70 - 300
> 300 - 500
> 500 - 700
> 700 - 1,000
> 1,000 - 2,000
> 2,000
general direction of groundwater flow
4 2 0 4 Feet
Cis-1,2-dichloroethene Concentrations for Plot #1
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Figure
5-12a
Guelph
14-Oct-09

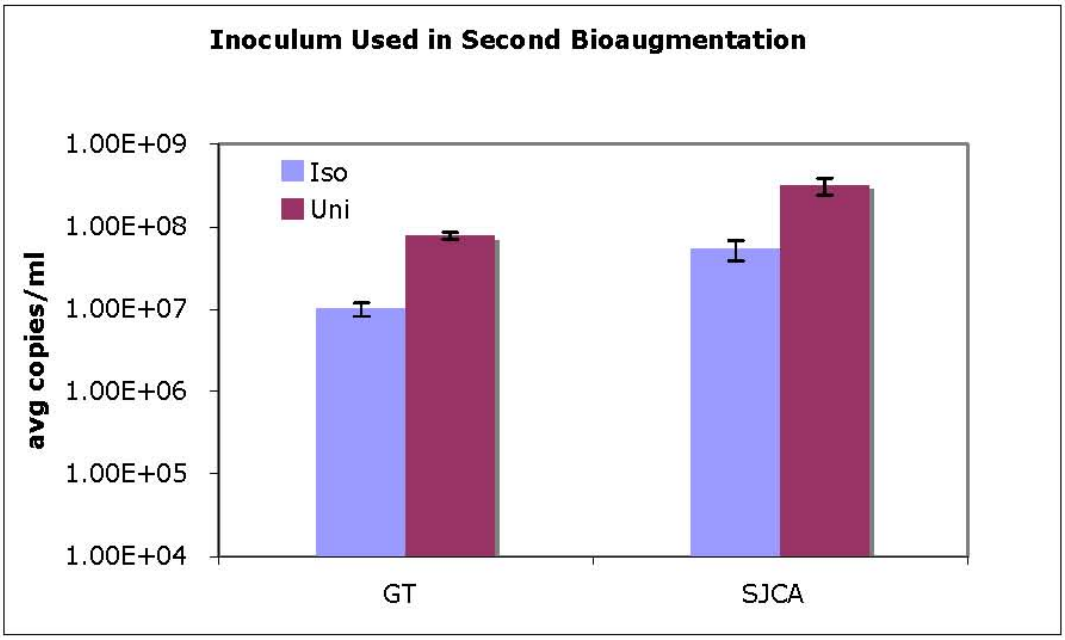

N
MW-06
650
MW-12
610
MW-11
640
MW-05
770
IW-02
560
MW-14
650
MW-13
990
MW-04
890
Baseline
October 2008
MW-06
480
MW-12
530
MW-11
500
MW-05
580
IW-02
580
MW-14
600
MW-13
610
MW-04
980
Month 1
December 2008
MW-06
590
MW-12
540
MW-11
510
MW-05
600
IW-02
540
MW-14
560
MW-13
620
MW-04
800
Month 2
January 2009
MW-06
540
MW-12
610
MW-11
860
MW-05
720
IW-02
510
MW-14
450
MW-13
940
MW-04
1,000
Month 3
February 2009
MW-06
580
MW-12
520 J
MW-11
580 J
MW-05
360
IW-02
420
MW-14
380 J
MW-13
570 J
MW-04
810
Month 4
March 2009
MW-06
460
MW-12
430
MW-11
650
MW-05
320
IW-02
430
MW-14
330
MW-13
600
MW-04
730
Month 5
April 2009
MW-06
790
MW-12
750
MW-11
890
MW-05
500
IW-02
710
MW-14
360
MW-13
1,200
MW-04
870
Month 6
May 2009
Legend
Concentration (µg/L)
≤ 70
> 70 - 300
> 300 - 500
> 500 - 700
> 700 - 1,000
> 1,000 - 2,000
> 2,000
general direction of groundwater flow
4 2 0 4 Feet
Cis-1,2-dichloroethene Concentrations for Plot #2
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Figure
5-12b
Guelph
14-Oct-09


N
MW-16
310
MW-15
160
IW-03
100
Baseline
October 2008
MW-16
250
MW-15
190
IW-03
39
Month 1
December 2008
MW-16
190
MW-15
160
IW-03
110
Month 2
January 2009
MW-16
300
MW-15
150
IW-03
100
Month 3
February 2009
MW-16
150 J
MW-15
160 J
IW-03
150
Month 4
March 2009
MW-16
180
MW-15
150
IW-03
71
Month 5
April 2009
MW-16
220
MW-15
170
IW-03
75
Month 6
May 2009
Legend
Concentration (µg/L)
≤ 70
> 70 - 300
> 300 - 500
> 500 - 700
> 700 - 1,000
> 1,000 - 2,000
> 2,000
general direction of groundwater flow
4 2 0 4 Feet
Cis-1,2-dichloroethene Concentrations for Plot #3
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Guelph
14-Oct-09
Figure
5-12c


N
MW-18
550
MW-17
820
IW-04
42
Baseline
October 2008
MW-18
190
MW-17
220
IW-04
15
Month 1
December 2008
MW-18
190
MW-17
290
IW-04
13
Month 2
January 2009
MW-18
460
MW-17
900
IW-04
34
Month 3
February 2009
MW-18
240
MW-17
260 J
IW-04
17 J
Month 4
March 2009
MW-18
110
MW-17
190
IW-04
41
Month 5
April 2009
MW-18
150
MW-17
660
IW-04
130
Month 6
May 2009
Legend
Concentration (µg/L)
≤ 70
> 70 - 300
> 300 - 500
> 500 - 700
> 700 - 1,000
> 1,000 - 2,000
> 2,000
general direction of groundwater flow
4 2 0 4 Feet
Cis-1,2-dichloroethene Concentrations for Plot #4
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Figure
5-12d
Guelph
14-Oct-09


N
MW-18
26
MW-17
8.5
IW-04
2.3
Baseline
October 2008
MW-18
15
MW-17
15
IW-04
1.8
Month 1
December 2008
MW-18
35
MW-17
22
IW-04
5.0 U
Month 2
January 2009
MW-18
61
MW-17
25 U
IW-04
5.0 U
Month 3
February 2009
MW-18
51
MW-17
18 J
IW-04
5.0 UJ
Month 4
March 2009
MW-18
33
MW-17
21
IW-04
5.0 U
Month 5
April 2009
MW-18
40
MW-17
31
IW-04
8.8
Month 6
May 2009
Legend
Concentration (µg/L)
Non-Detect
≤ 2
> 2 - 20
> 20 - 40
> 40 - 60
> 60
general direction of groundwater flow
4 2 0 4 Feet
Vinyl Chloride Concentrations for Plot #4
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Guelph
14-Oct-09
Figure
5-13





DRAFT

qPCR Results for Inoculum Cultures
Site 21, St. Julien's Creek Annex, Chesapeake, VA

Geosyntec consultants

Figure 5-14

Guelph | September 2009

P:\PM\Projects\IW0207-ESTCP JS666 Biocug\Task 7 - Reporting\Final Report\Figures\[Figure 5-13 - qPCR results for inoculum cultures.xls]Figure 5-2

Probe Assay – Monitoring JS666 Transport

qPCR data are presented graphically for wells in Plots #1 to #4 over the course of the demonstration on Figures 5-15a to 5-15d, respectively. The qPCR data represents ISO probe values (if only ISO data were collected) or the lower of ISO and CMO values (if both probes were used). A table of the plotted data can be found in Appendix E (Table E-5) along with figures illustrating quantitative data for ISO, CMO and UNI probes (Figures E-1 through E-7).

In Figures 5-15a to 5-15d, qPCR results are coded for each sampling location and event as follows: "0" for nondetectable levels; "+" for results considered detectable, but not quantifiable (i.e., reasonable agreement in most qPCR plate-wells, but less than 20 copies/rxn); "++" for quantifiable levels below 10^4 copies per mL ($20 \leq$ copies/rxn ≤ 60); and "+++" for levels above 10^4 per mL. Note that because a single "+" suggests detection but not quantification, there are results in the Table that are coded "+" that do not appear on the quantitative charts plotted in Figures E-1 through E-7.

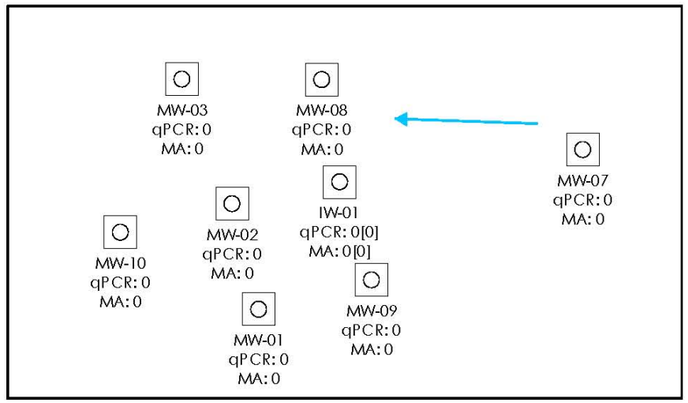
For all of the plots, there is no evidence of JS666 during the baseline sampling event (October 2008) prior to the addition of JS666 and buffer (and oxygen in some cases). In general, there are almost no qPCR detections in the control plots (#3 and #4) where no JS666 was added, with the exception of a few sporadic low level qPCR hits. Likewise, there are no qPCR detections in upgradient wells MW-07 or MW-11 (with the exception of one low level hit in MW-11 in January 2009). Taken together these data indicate that there is no significant native population of JS666.

The qPCR data for Bioaugmentation Plot #1 is shown in Figure 5-15a. The highest levels were generally observed in January 2009 (two months after the first bioaugmentation) and typically levels were highest in the MW-08, which is transgradient to the injection well. qPCR data show that JS666 bacteria have migrated at least 6 ft downgradient.

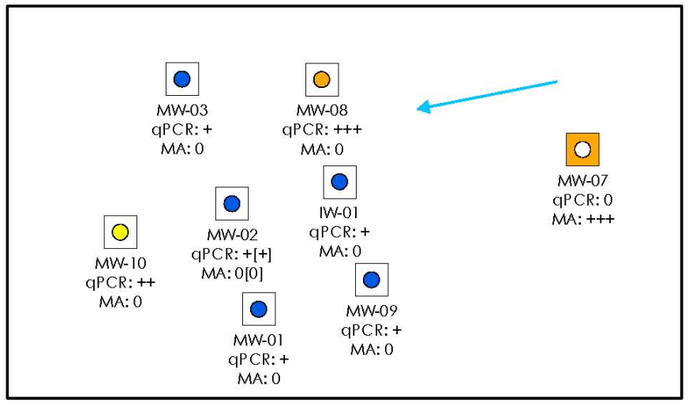
The qPCR data for Bioaugmentation Plot #2 is presented in Figure 5-15b. The best distribution of JS666 was generally observed in March 2009 (one month after the second bioaugmentation). The qPCR counts declined in the months following. qPCR data show that JS666 bacteria have migrated at least 8 feet downgradient. Growth is not clearly observed throughout the demonstration either due to oxygen limitation or the cells washing out of the test area.

Microcosm Assay - Monitoring

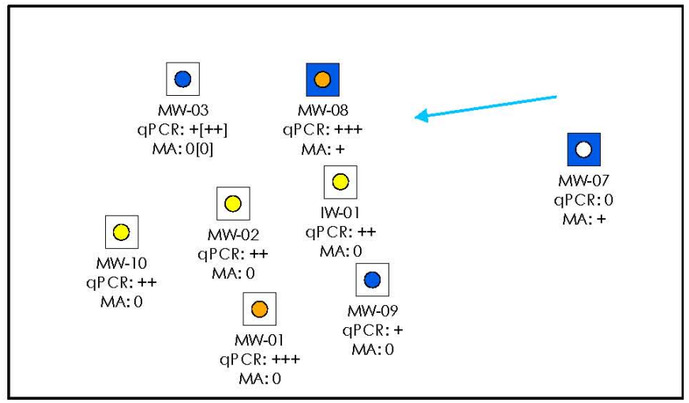
For the seven sampling events, groundwater samples were collected from each of the wells, along with two quality-control duplicates. These groundwater samples were used to conduct microcosm assays in triplicate. Generally only two of the three microcosm replicates were sampled, unless there was significant variability (in which case the third replicate would be sampled as a tie-breaker).Two examples of such assays (showing data from two replicates) from the April sampling event are presented in Figure 5-16: MW-11 is an upgradient well in



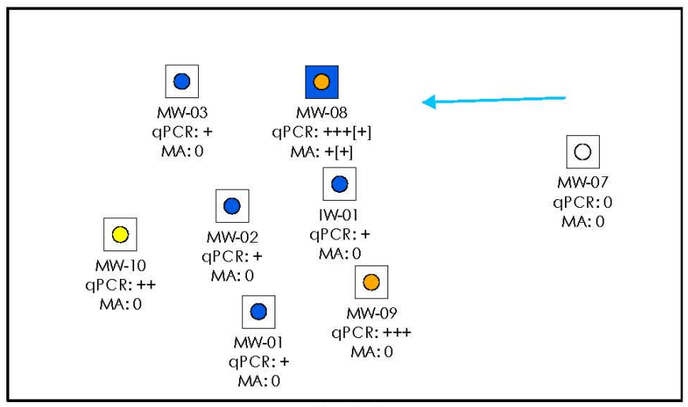
Baseline
October 2008



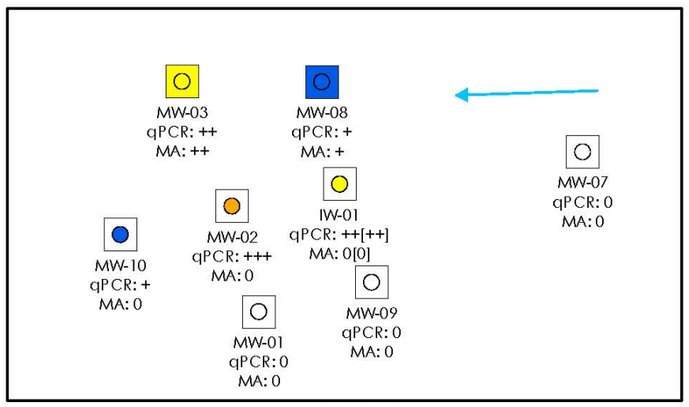
Month 1
December 2008



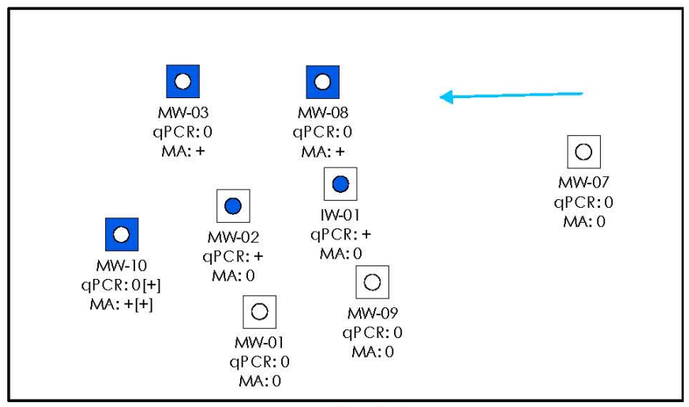
Month 2
January 2009



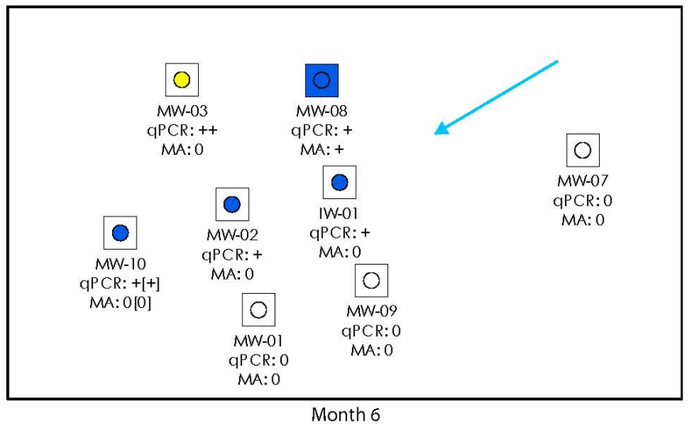
Month 3
February 2009



Month 4
March 2009



Month 5
April 2009



Month 6
May 2009

Legend

qPCR

- 0 (Non-Detect)
- + (<3.3E03/mL)
- ++ (<E04/mL)
- +++ (>E04/mL)

Microcosm Activity

- 0 (No degr within 40 d)
- + (>90% degr within 40 d)
- ++ (>90% degr within 30 d)
- +++ (>90% degr within 20 d)

general direction of groundwater flow

N

4 2 0 4 Feet

qPCR and Microcosm Activity for Plot #1

Site 21, St. Julien's Creek Annex, Chesapeake, VA

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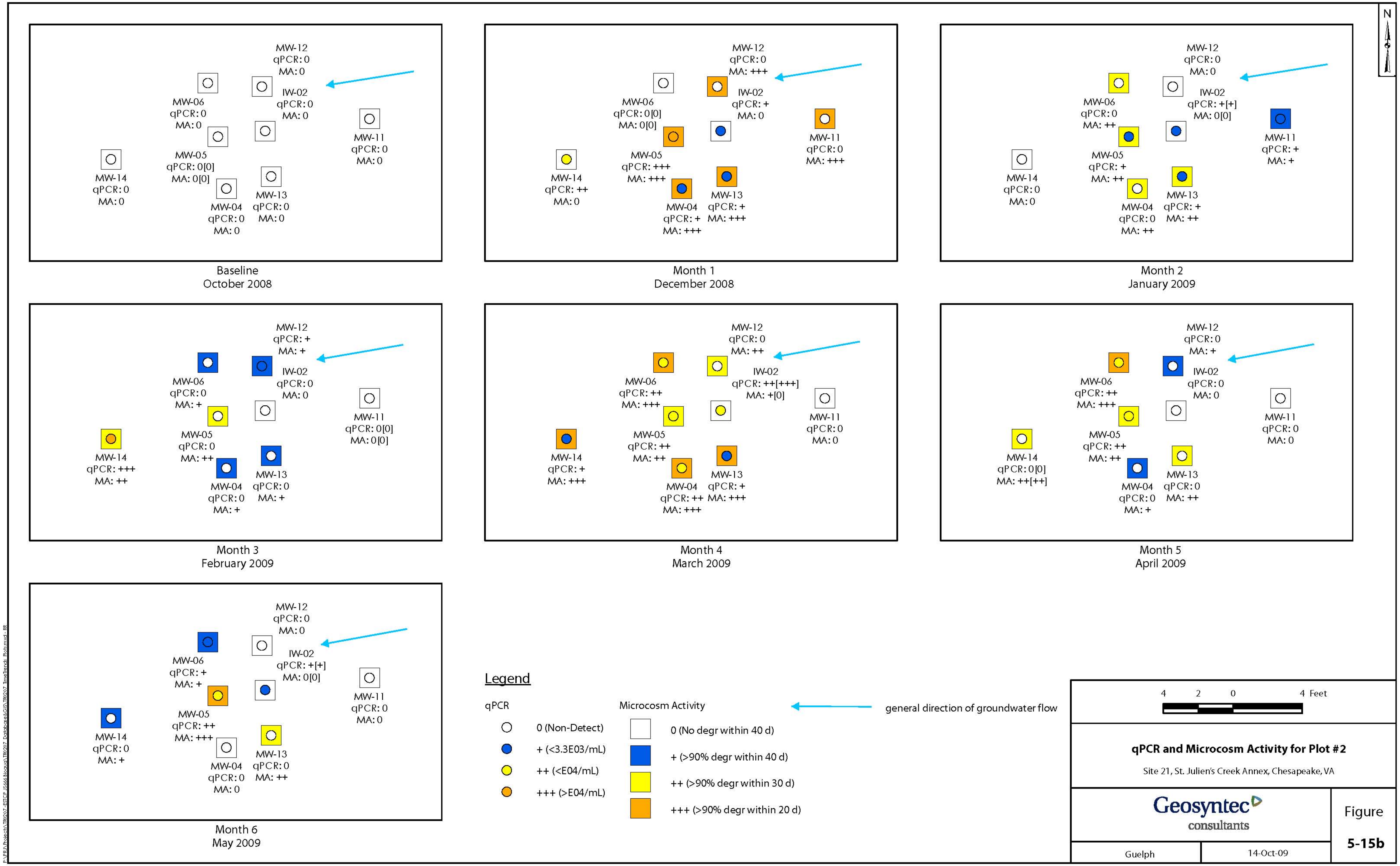
Figure **5-15a**



MW-12 qPCR: 0 MA: 0
IW-02 qPCR: 0 MA: 0
MW-06 qPCR: 0 MA: 0
MW-05 qPCR: 0[0] MA: 0[0]
MW-11 qPCR: 0 MA: 0
MW-14 qPCR: 0 MA: 0
MW-04 qPCR: 0 MA: 0
MW-13 qPCR: 0 MA: 0
Baseline
October 2008
MW-12 qPCR: 0 MA: +++
IW-02 qPCR: + MA: 0
MW-06 qPCR: 0[0] MA: 0[0]
MW-05 qPCR: +++ MA: +++
MW-11 qPCR: 0 MA: +++
MW-14 qPCR: ++ MA: 0
MW-04 qPCR: + MA: +++
MW-13 qPCR: + MA: +++
Month 1
December 2008
MW-12 qPCR: 0 MA: 0
IW-02 qPCR: +[+] MA: 0[0]
MW-06 qPCR: 0 MA: ++
MW-05 qPCR: + MA: ++
MW-11 qPCR: + MA: +
MW-14 qPCR: 0 MA: 0
MW-04 qPCR: 0 MA: ++
MW-13 qPCR: + MA: ++
Month 2
January 2009
MW-12 qPCR: + MA: +
IW-02 qPCR: 0 MA: 0
MW-06 qPCR: 0 MA: +
MW-05 qPCR: 0 MA: ++
MW-11 qPCR: 0[0] MA: 0[0]
MW-14 qPCR: +++ MA: ++
MW-04 qPCR: 0 MA: +
MW-13 qPCR: 0 MA: +
Month 3
February 2009
MW-12 qPCR: 0 MA: ++
IW-02 qPCR: ++[+++] MA: +[0]
MW-06 qPCR: ++ MA: +++
MW-05 qPCR: ++ MA: ++
MW-11 qPCR: 0 MA: 0
MW-14 qPCR: + MA: +++
MW-04 qPCR: ++ MA: +++
MW-13 qPCR: + MA: +++
Month 4
March 2009
MW-12 qPCR: 0 MA: +
IW-02 qPCR: 0 MA: 0
MW-06 qPCR: ++ MA: +++
MW-05 qPCR: ++ MA: ++
MW-11 qPCR: 0 MA: 0
MW-14 qPCR: 0[0] MA: ++[++]
MW-04 qPCR: 0 MA: +
MW-13 qPCR: 0 MA: ++
Month 5
April 2009
MW-12 qPCR: 0 MA: 0
IW-02 qPCR: +[+] MA: 0[0]
MW-06 qPCR: + MA: +
MW-05 qPCR: ++ MA: +++
MW-11 qPCR: 0 MA: 0
MW-14 qPCR: 0 MA: +
MW-04 qPCR: 0 MA: 0
MW-13 qPCR: 0 MA: ++
Month 6
May 2009
Legend
qPCR
0 (Non-Detect)
+ (<3.3E03/mL)
++ (<E04/mL)
+++ (>E04/mL)
Microcosm Activity
0 (No degr within 40 d)
+ (>90% degr within 40 d)
++ (>90% degr within 30 d)
+++ (>90% degr within 20 d)
general direction of groundwater flow
N
4 2 0 4 Feet
qPCR and Microcosm Activity for Plot #2
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Guelph
14-Oct-09
Figure
5-15b

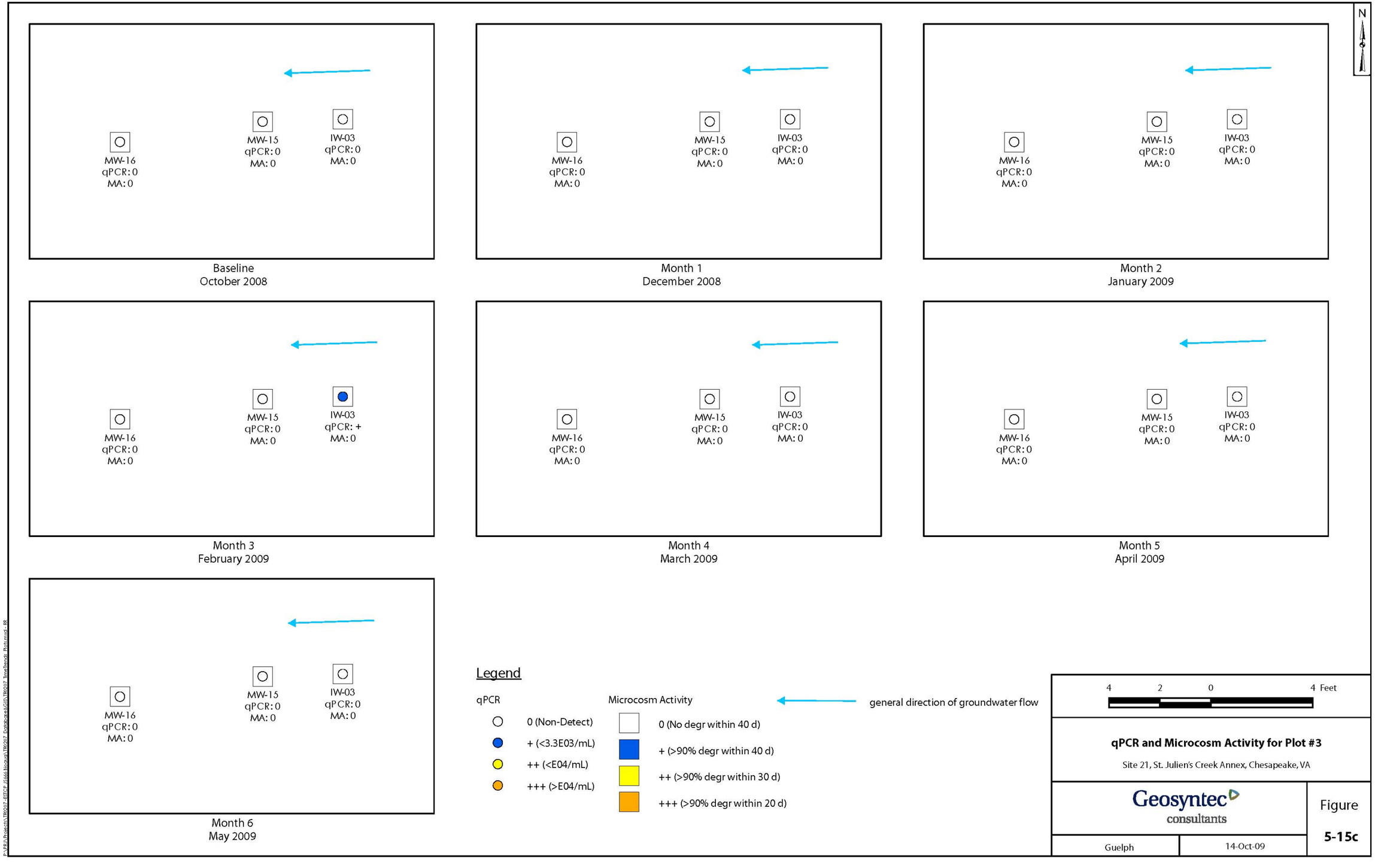

N
MW-16
qPCR: 0
MA: 0
MW-15
qPCR: 0
MA: 0
IW-03
qPCR: 0
MA: 0
Baseline
October 2008
Month 1
December 2008
Month 2
January 2009
qPCR: +
Month 3
February 2009
Month 4
March 2009
Month 5
April 2009
Month 6
May 2009
Legend
qPCR
0 (Non-Detect)
+ (<3.3E03/mL)
++ (<E04/mL)
+++ (>E04/mL)
Microcosm Activity
0 (No degr within 40 d)
+ (>90% degr within 40 d)
++ (>90% degr within 30 d)
+++ (>90% degr within 20 d)
general direction of groundwater flow
4 2 0 4 Feet
qPCR and Microcosm Activity for Plot #3
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Guelph
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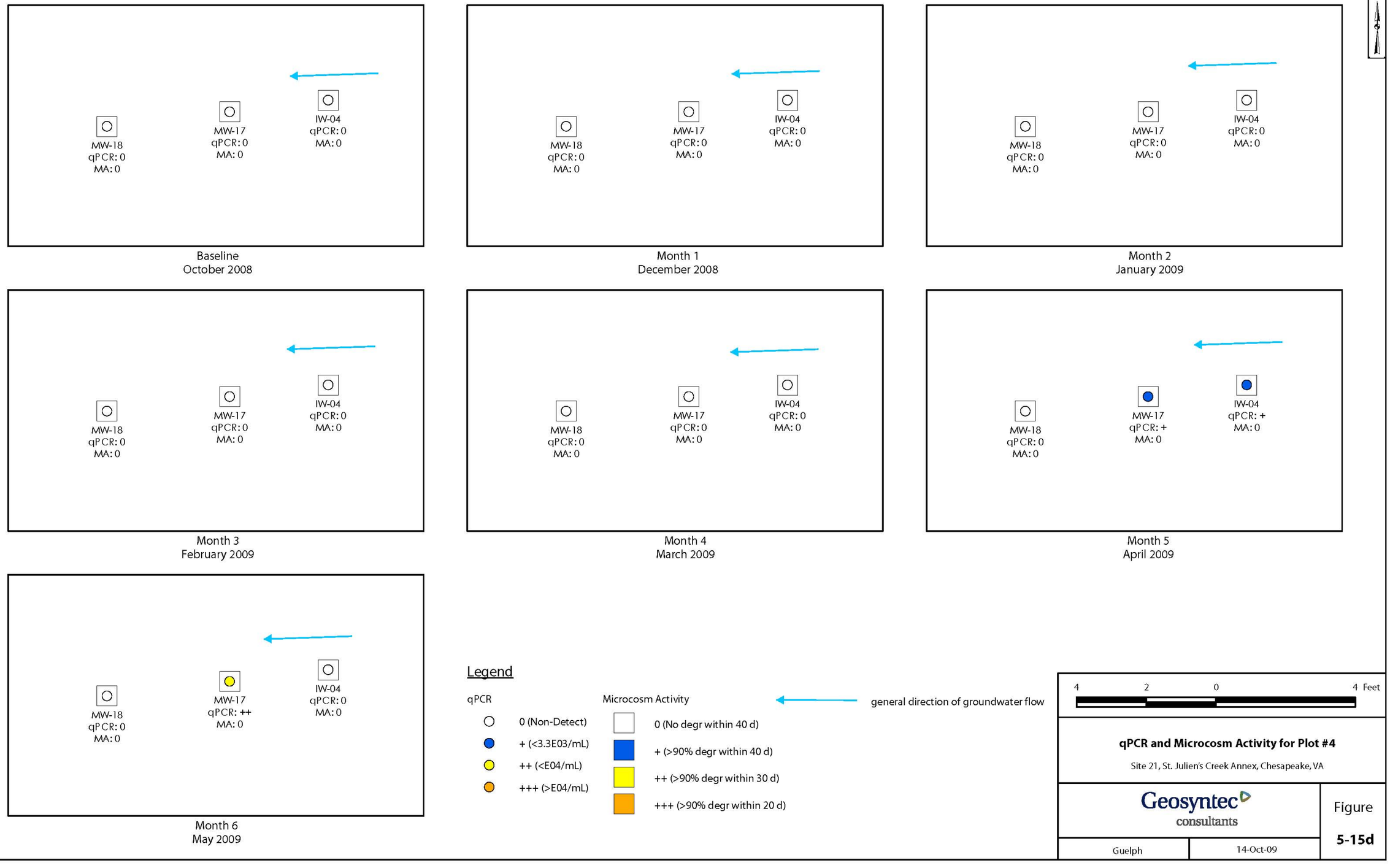
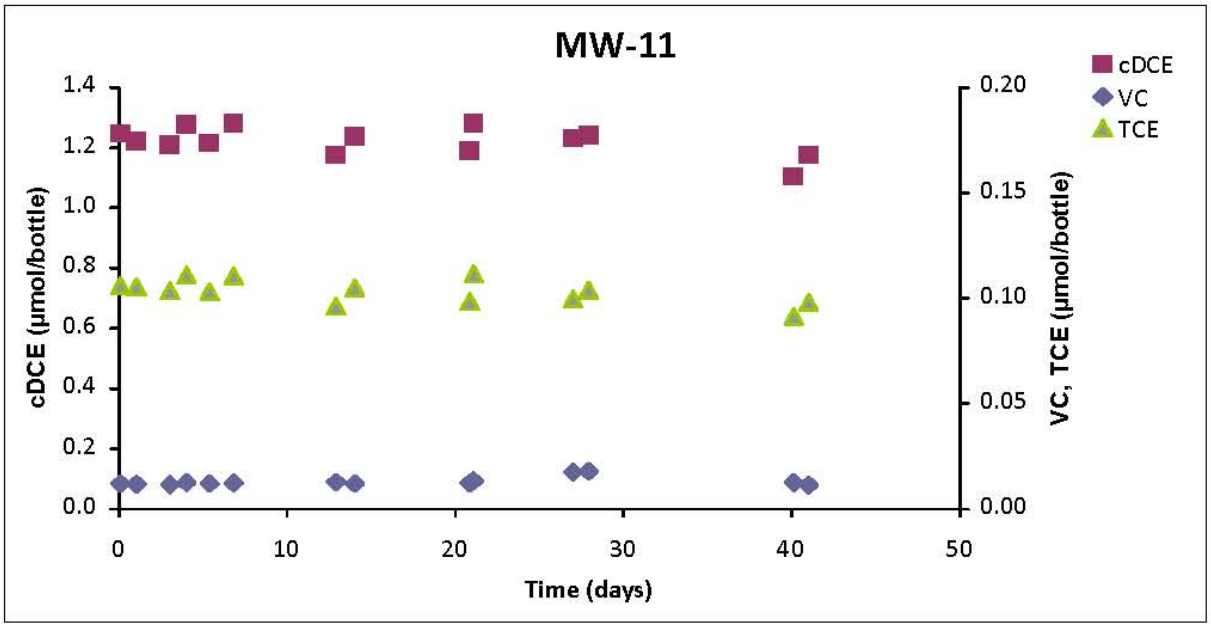
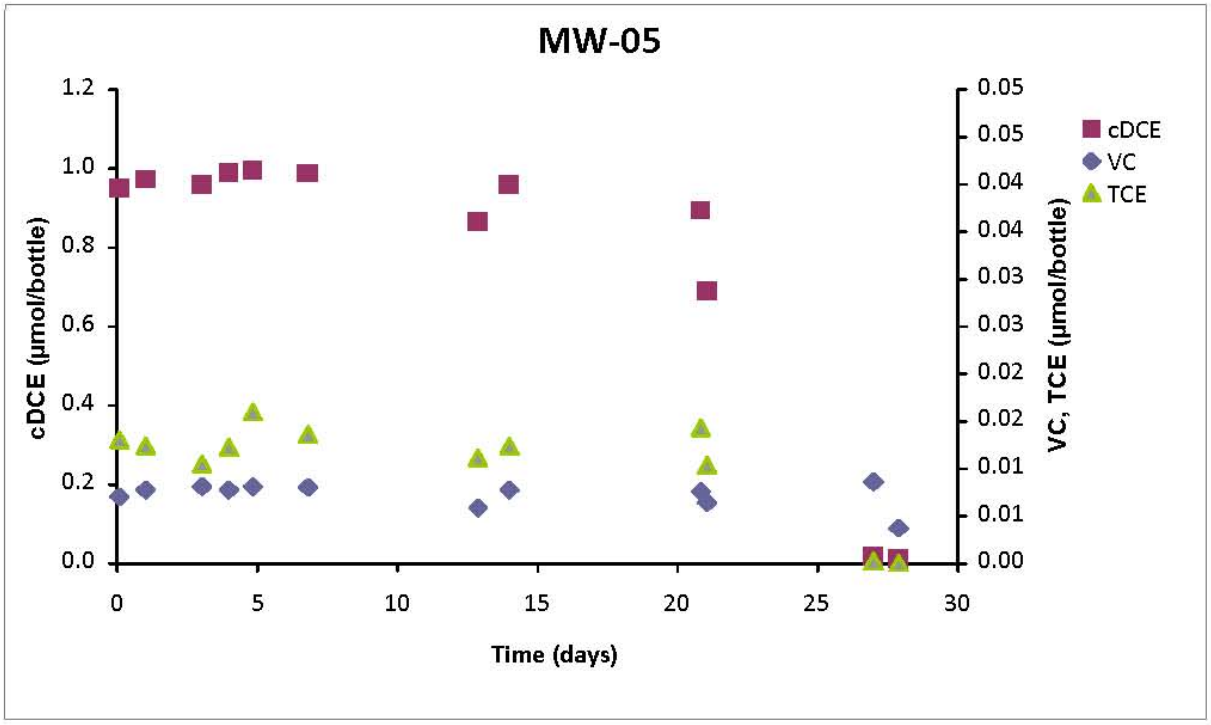
Figure
5-15c


N
MW-18
qPCR: 0
MA: 0
MW-17
qPCR: 0
MA: 0
IW-04
qPCR: 0
MA: 0
Baseline
October 2008
Month 1
December 2008
Month 2
January 2009
MW-17
qPCR: +
MA: 0
IW-04
qPCR: +
MA: 0
Month 3
February 2009
Month 4
March 2009
Month 5
April 2009
MW-17
qPCR: ++
MA: 0
Month 6
May 2009
Legend
qPCR
0 (Non-Detect)
+ (<3.3E03/mL)
++ (<E04/mL)
+++ (>E04/mL)
Microcosm Activity
0 (No degr within 40 d)
+ (>90% degr within 40 d)
++ (>90% degr within 30 d)
+++ (>90% degr within 20 d)
general direction of groundwater flow
4 2 0 4 Feet
qPCR and Microcosm Activity for Plot #4
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Guelph
14-Oct-09
Figure
5-15d





P:\PRA\Projects\TR0207 - BSTCP JA666 Biocurg\Task 7 - Reporting\Final Report\Figures\[Figure 5-16 - Example Microcosm VOC Results.xls]Figure 5-2

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Exemplary Microcosm VOC Results
Site 21, St. Julien's Creek Annex, Chesapeake, VA

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Figure 5-16

Bioaugmentation Plot #2, and MW-05 is the well immediately downgradient of the injection well in Plot #2. For MW-11, there is no marked change in cDCE, VC, or TCE levels over more than 40 days of monitoring, which is typical of an assay with "negative" activity. For MW-05, the concentration of all the VOCs declines significantly by day 27. These results are typical of an assay judged "++" (i.e., it evidenced >90% degradation of cDCE within 30 days). Note that virtually all microcosms that showed degradation of cDCE also showed concomitant degradation of TCE (and usually also of VC, though that is less evident in this particular example of MW-05).

Results of the microcosm assays for each of the plots over the course of the demonstration are shown in Figures 5-15a through 5-15d. Microcosm activity results have been coded as follows: "0" if no cDCE degradation occurred over 40 days of monitoring; "+" if cDCE was degraded within 40 days; "++" if within 30 days; "+++" if within 20 days; and "++++" if within 10 days. For this purpose, "degradation" was considered to be greater than 90% disappearance of cDCE.

Through examination of Figures 5-15a through 5-15d, it is evident that no microcosm activity was evident in any of the plots prior to bioaugmentation and buffer addition (Baseline, October 2008). Likewise, there is no microcosm activity in samples collected from wells in the Control Plots #3 and #4 during any of the sampling events.

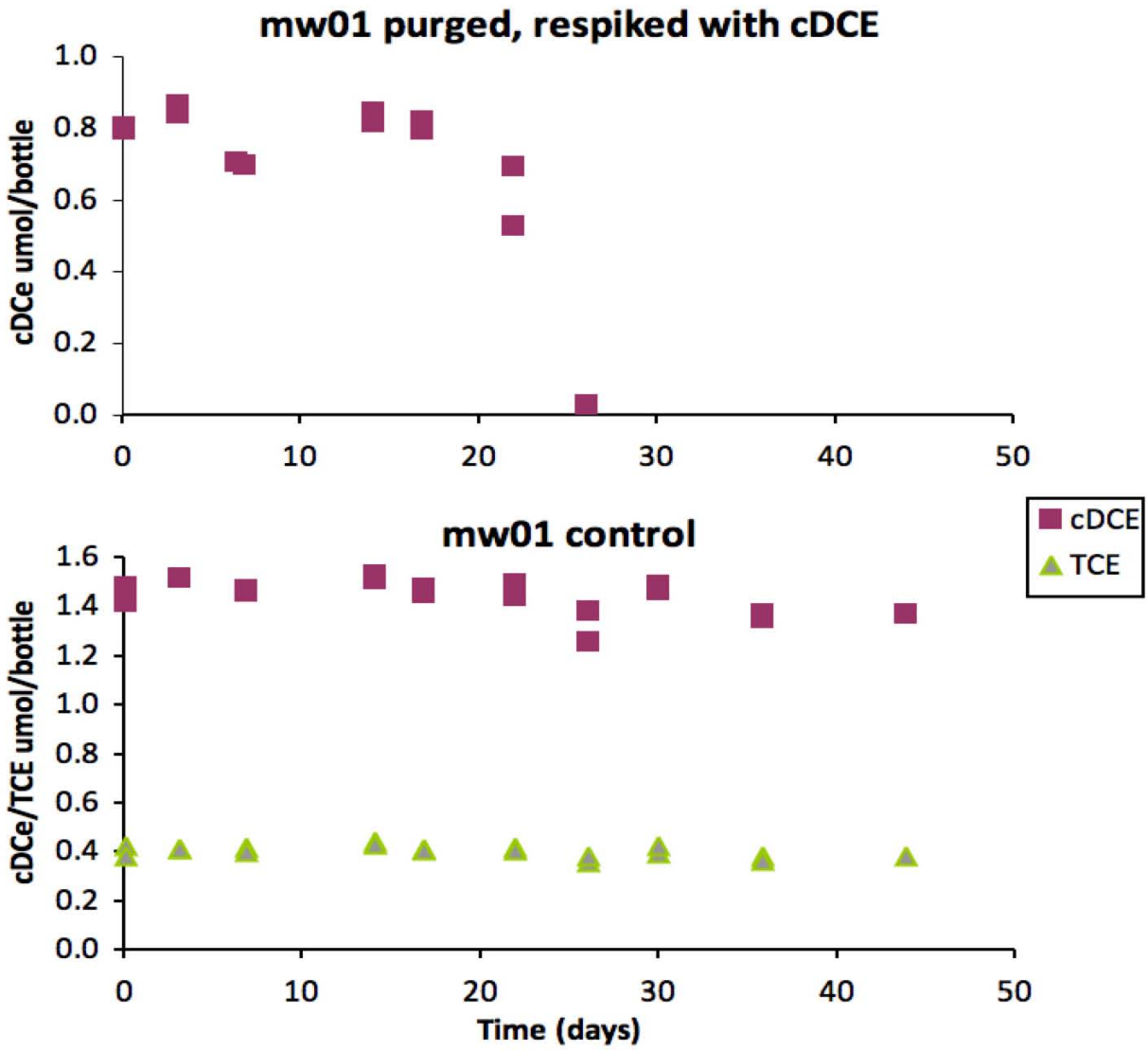
In Bioaugmentation Plot #1, although JS666 was typically present according to the qPCR assays, microcosm activity was not generally evident or evident at very low levels in downgradient wells. The exception to this was the activity measured in MW-03 during the March 2009 sampling event (> 90% degradation within 30 days), which corresponded to a JS666 density of between 3.3 x 10^3 and 10^4 CFU/mL. The low microcosm activity is likely attributable to the high TCE concentrations (greater than 1000 ug/L) in Plot #1. High TCE concentrations (i.e., greater than 500 ug/L) can inhibit cDCE biodegradation as illustrated in additional microcosm studies discussed below in the section *Microcosm Assays – Additional*.

In Bioaugmentation Plot #2, the highest microcosm activity was generally observed one month following each of the two bioaugmentations (in December 2008 and then in March 2009) and then decreased in the months following. Higher microcosm activity was observed in groundwater samples from Bioaugmentation Plot #2 compared to Bioaugmentation Plot #1, likely due to the lower concentrations of TCE in groundwater samples in Bioaugmentation Plot #2. Note that isotopic analyses indicated more biodegradation in Bioaugmentation Plot #1 relative to Bioaugmentation Plot #2; so microbial activity may have been higher in Plot #1 than measured in the laboratory using groundwater samples, which likely had lower levels of JS666 than the surrounding aquifer matrix.

Microcosm Assays – Additional

In parallel SERDP-sponsored studies, TCE was inhibitory to JS666 at concentrations above 4 µM (500 µg/L) at low JS666 levels. Unfortunately at SJCA, the levels of TCE in some

locations of Bioaugmentation Plot #1 exceeded 1,000 µg/L. To test whether TCE inhibited cDCE degradation in Bioaugmentation Plot #1, microcosms were constructed from MW-01 samples (January sampling event). Control microcosms (using groundwater "as-received") and air-stripped microcosms (which were stripped of VOCs and then re-spiked with cDCE only) were run in parallel. The control MW-01 sample had a TCE concentration of about 8 uM (1050 µg/L). The results are presented in Figure 5-17 and suggest that high TCE levels may have adversely impacted microcosm assays from Bioaugmentation Plot#1 and, by extension, may have adversely impacted the degree of cDCE degradation in the field.



P:\PR\Projects\TR0207 - ESTCP .ISSdd Bioaug\Task 7 - Reporting\Final Report\Figures\[Figure 5-16 - VOC Results from Parallel Microcosm Studies.xls]Figure 5-2

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VOC Results from Parallel Microcosm Studies
Site 21, St. Julien's Creek Annex, Chesapeake, VA

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Figure 5-17

6.0 PERFORMANCE ASSESSMENT

6.1 REDUCTION IN cDCE CONCENTRATIONS

6.1.1 Qualitative

A key performance objective was greater reductions of cDCE concentrations in bioaugmentation plots versus control plots. To evaluate this objective, cDCE data from bioaugmentation plots were compared to data from control plots and from background (upgradient) wells. cDCE data in Bioaugmentation Plots #1 and #2 and in Control Plots #3 and #4 are shown in Figures 5-12a to 5-12d and summarized in Table 6-1. Comparison of cDCE concentrations over time in the bioaugmented plots to the control plots reveals some reduction in cDCE in several wells (e.g., MW-1, MW-2, MW-3, MW-5, MW-6, MW-10, MW-12, and MW-14), indicating the effectiveness of JS666 bioaugmentation. Isotopic enrichment in groundwater samples in the bioaugmented wells compared to the upgradient and Control Plots #3 and #4 wells further corroborates the effect of JS666 bioaugmentation on cDCE degradation as discussed in Section 5.7.4. Therefore, greater cDCE reductions were observed in many of the wells in the bioaugmented plots compared to the control plots. cDCE biodegradation was likely limited by lack of oxygen in Bioaugmentation Plot #2 and inhibited by high levels of TCE in Bioaugmentation Plot #1 as discussed in Section 5.7.6.

6.1.2 Quantitative

When cDCE concentration reductions were quantitatively evaluated, the objective was to achieve greater than 75% reduction in cDCE in bioaugmentation plots over background concentrations and twice the reduction of cDCE concentrations in bioaugmented plots versus control plots.

Table 6-1 presents percent removals of cDCE based on average upgradient and downgradient concentrations. Although reductions in average cDCE concentrations of up to 44% were observed in Bioaugmentation Plot #1 and up to 25% were observed in Bioaugmentation Plot #2 relative to average upgradient cDCE concentrations, the objective of a 75% reduction was not achieved. The reduction was also evaluated by plotting normalized concentrations in each well for a selected event (April 2009) relative to baseline concentrations for both bioaugmentation plots and control plots as shown in Figures 6-1a and 6-1b. Note that the cDCE concentrations from the May 2009 sampling event were not used because of problems with the air cylinder supplying the Waterloo Emitter. None of the wells in either bioaugmentation plot showed cDCE reductions of 75% or more (i.e., a normalized C/Co of 0.25 or less) relative to baseline. Furthermore, although cDCE concentrations declined in many of the bioaugmentation wells (i.e., all downgradient wells in Plot#2); the reductions were not typically twice that observed in the control plot wells.

In addition to high TCE concentrations in Bioaugmentation Plot #1, the reason for not meeting the performance objective may be due to the difficulty in achieving good dissolved

TABLE 6-1
% Removal of cDCE in Wells in Bioaugmentation Plots #1 and #2
Site 21, St. Julien's Creek Annex, Chesapeake, VA

Plot #1

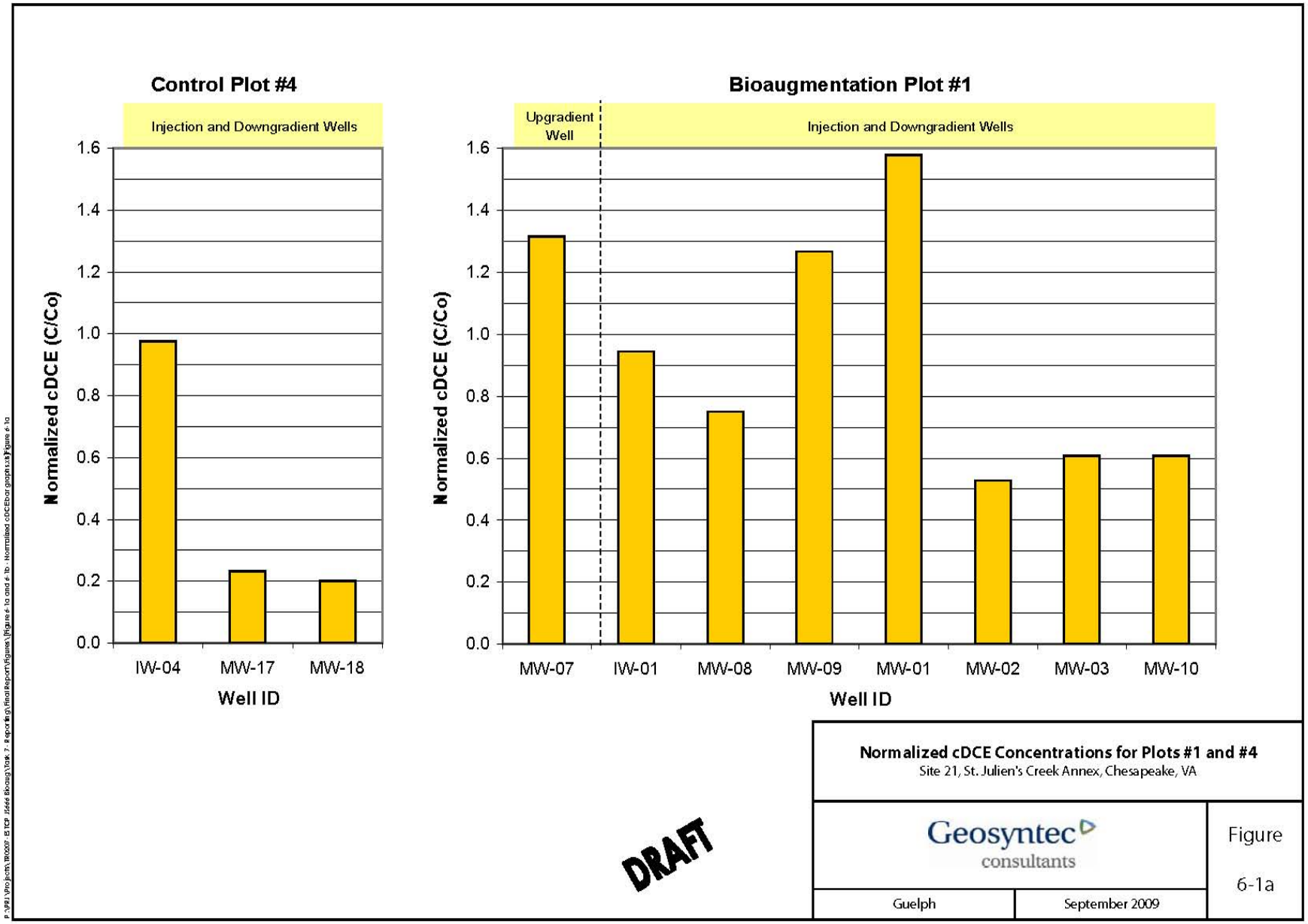
Well ID	Average cDCE Concentration (ug/L)	% Removal
MW-07 (upgradient)	2,533	--
IW-01	1,459	42
MW-01	1,420	44
MW-02	1,580	38
MW-03	2400	42
MW-08	2350	7
MW-09	2400	23
MW-10	1,800	29

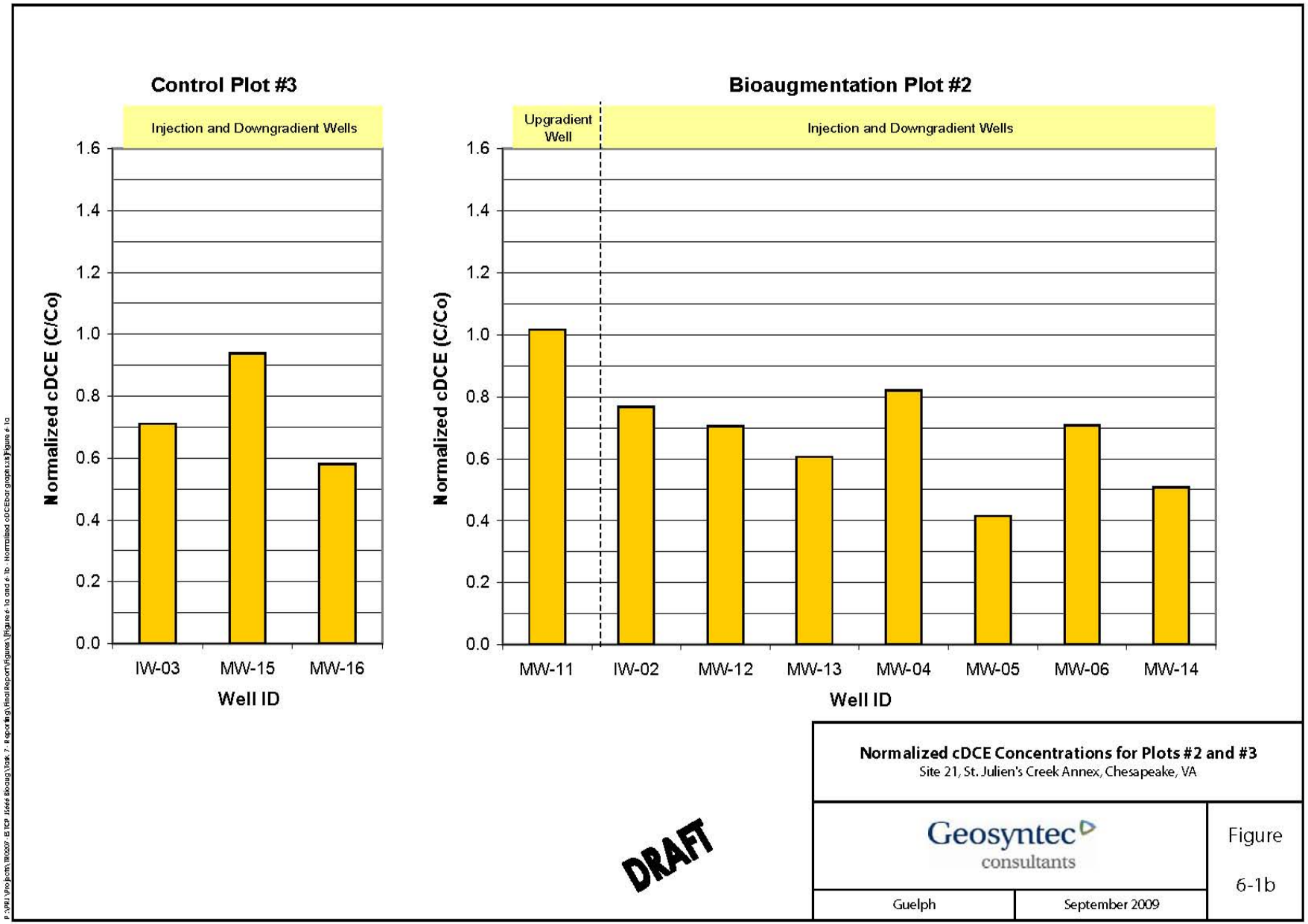
Plot #2

Well ID	Average cDCE Concentration (ug/L)	% Removal
MW-11 (upgradient)	620	--
IW-02	497	20
MW-04	864	-39
MW-05	516	17
MW-06	532	14
MW-12	526	15
MW-13	674	-9
MW-14	464	25

Notes:

Average cDCE concentrations were calculated from October 2008 to April 2009 for upgradient wells and from December 2008 to April 2009 for downgradient wells.


Control Plot #4
Injection and Downgradient Wells
Normalized cDCE (C/Co)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
IW-04
MW-17
MW-18
Well ID
Bioaugmentation Plot #1
Upgradient Well
Injection and Downgradient Wells
Normalized cDCE (C/Co)
MW-07
IW-01
MW-08
MW-09
MW-01
MW-02
MW-03
MW-10
Well ID
DRAFT
Normalized cDCE Concentrations for Plots #1 and #4
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Figure
6-1a
Guelph
September 2009


Control Plot #3
Injection and Downgradient Wells
Normalized cDCE (C/Co)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
IW-03
MW-15
MW-16
Well ID
Bioaugmentation Plot #2
Upgradient Well
Injection and Downgradient Wells
Normalized cDCE (C/Co)
MW-11
IW-02
MW-12
MW-13
MW-04
MW-05
MW-06
MW-14
Well ID
DRAFT
Normalized cDCE Concentrations for Plots #2 and #3
Site 21, St. Julien's Creek Annex, Chesapeake, VA
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Guelph
September 2009
Figure
6-1b

oxygen distribution. A diffusive gas emitter device (the Waterloo Emitter) was employed and elevated oxygen levels were generally limited to the vicinity of the injection well only. The emitter was supplied with compressed air instead of compressed oxygen because JS666 is sensitive to oxygen levels above 10 mg/L. Air sparging might have been more effective in distributing oxygen a further distance from the injection well. This approach was initially discounted as preliminary lithologic data had suggested the subsurface was heterogeneous and good oxygen distribution would not be achieved. Furthermore, the ambient dissolved oxygen concentrations were very low in Bioaugmentation Plot #2, which undoubtedly influenced the performance in that plot.

6.2 GROWTH AND SPATIAL DISTRIBUTION OF JS666

The objective associated with the growth and distribution of JS666 was to determine the extent of transport of JS666 away from the injection well. JS666 was enumerated in groundwater samples using two molecular probes (one based on the isocitrate lyase gene and one based on the cyclohexanone monooygenase gene). In addition, JS666 activity and presence was also evaluated through microcosm assays conducted using groundwater from the wells in each of the plots. Successful distribution was indicated by the presence and activity of JS666 in bioaugmented plots but not in control plots or background wells. Growth of JS666 was demonstrated by observing higher counts of JS666 with time in bioaugmented plots.

Following bioaugmentation, qPCR and microcosm results demonstrated in-situ survival and activity over the course of the demonstration in the bioaugmentation plots (Figures 5-15a and 5-15b). Though the levels of JS666 were low (i.e., 3×10^3 to 10^4 CFU/mL), they were adequate to effect cDCE degradation, if suitable environmental conditions (adequate oxygen, pH and absence of inhibitory levels of TCE) were present. In general, there were very few qPCR detections in the control plots where no JS666 was added. Likewise there were no qPCR detections in either of the upgradient wells (MW-7 and MW-11), except for one instance of a 3.3×10^3 CFU/mL detection in MW-11. Thus, the pilot tests were successful in demonstrating the spread of the JS666 organisms in the bioaugmented plots. It was difficult to tell whether growth was occurring because bacterial densities did not consistently increase over time.

The microcosms were apparently more sensitive detectors of JS666 than was qPCR – i.e., positive microcosm activity (if one uses 40 days to degradation as measure) was observed in downgradient samples in many instances where qPCR was negative (Figures 5-15a and Figure 5-15b). The fact that such positive microcosm results occurred only in samples from locations downgradient of bioaugmentation (rather than in control plots, for example) is meaningful. It should be noted that microcosm assays were conducted at 22°C (compared to 17°C of groundwater) and were not oxygen-limited. On the other hand, field D.O. levels were quite low. These results demonstrated that the JS666 cells were transported through the subsurface and maintained their activity.

6.3 IMPACT OF OXYGEN LEVELS ON GROWTH AND DEGRADATION RATES

To evaluate the impact of oxygen levels on growth and degradation rates, we originally planned to compare the impact of higher oxygen levels (relative to ambient) on the growth of JS666 and rate of cDCE degradation between the bioaugmented plots with similar VOC concentrations. Despite the higher TCE concentrations in Bioaugmentation Plot #1, more biodegradation was observed as illustrated by the higher degree of $\delta^{13}C$ enrichment in Bioaugmentation Plot #1 as discussed in Section 5.7.4. The higher degree of $\delta^{13}C$ enrichment may have been due to more biodegradation as a result of the added oxygen in IW-01. Both Bioaugmentation Plot #1 and Plot #2 had relatively low levels of JS666 according to qPCR measurements.

Therefore, the addition of oxygen appeared to increase the rate of cDCE degradation. However, increased JS666 growth rates could not definitely be identified in Plot #1 using qPCR data because the values were close to detection limits.

6.4 EASE OF USE

The ease of use of this technology was evaluated based on our experience in the field with the JS666 bacteria. Addition of the culture via injection wells was straightforward because it was an aerobic culture. Therefore, no special procedures were required to exclude oxygen during the injection. Because the native groundwater pH was low at the demonstration site, buffer was required. To distribute the buffer throughout the injection area, groundwater was extracted, amended with buffer, and then reinjected. Although the procedure was simple, it was time-consuming and needed to be repeated due to the soluble nature of the buffer employed. Aeration using the Waterloo Emitter was easy (only requiring change out of the compressed cylinder approximately monthly) but was not effective in distributing oxygen beyond the injection well. Ideally, JS666 should be employed in an aquifer with measurable dissolved oxygen (e.g., above 0.5-1 mg/L) or perhaps in an active recirculation system where oxygen can be metered into the injection stream continually.

6.5 COST COMPARISON

This bioaugmentation technology was compared to pump and treat over a 30 year time period. Results of the cost comparison are presented in Section 7.0. A present value cost-savings of 30-50% compared to pump and treat would represent a successful demonstration. The cost analysis shows a projected cost savings of 47%, assuming no aeration or buffering is required and sufficient oxygen is present in the groundwater naturally. Thus, under these assumptions, this technology is cost-effective when compared to pump and treat.

7.0 COST ASSESSMENT

This section presents the results of a cost assessment to implement enhanced in-situ bioremediation (EISB) of cDCE-impacted groundwater using JS666 as a bioaugmentation culture. Section 7.1 describes a cost model that was developed for the application of EISB using JS666; Section 7.2 presents an assessment of the cost drivers for the application of the technology; and Section 7.3 presents the results of an analysis of the cost model with a comparison to a conventional pump and treat system.

7.1 COST MODEL

A cost model was developed to assist remediation professionals in understanding costing implications associated with the JS666 EISB technology. The cost model identified the major cost elements required to implement the EISB approach at a typical site with cDCE-impacted shallow groundwater. A summary of the cost elements is presented in Table 7-1, along with the associated cost for each element as incurred during the current technology demonstration. The cost model focused on pilot-scale treatment of contaminated groundwater. Specifically excluded from consideration are the costs of pre-remediation investigations (e.g., plume delineation, risk determination, and related needs), treatability studies, permitting, source zone treatment, and post remediation and decommissioning.

Capital costs included design and planning activities, mobilization, and well installation. O&M costs included mobilization, the bioaugmentation culture, and amendment equipment and supplies (e.g., tracer, buffer and oxygen amendments). Performance monitoring costs included mobilization, field supplies, sampling equipment, laboratory analysis and reporting. Labor associated with the planning, procurement and implementation of all aspects of the EISB approach is also included.

While most of the identified cost elements are applicable to other remediation technologies, the groundwater amendments employed in this demonstration are fairly unique to the technology. The dose of the JS666 culture is relative to the size of the treatment area; so a larger treatment area will require a higher volume. The frequency and dose of other groundwater amendments (e.g., oxygen, pH buffer) will be dependent on site hydrology and geochemistry, but increased frequency and larger doses will ultimately result in higher operating costs.

7.2 COST DRIVERS

The costs to implement EISB of cDCE- impacted groundwater using JS666 will vary significantly from site to site. The key costs drivers are listed below, along with a brief discussion of their impact on cost.

TABLE 7-1: Cost Model for EISB Using JS666
Site 21, St. Julien's Creek Annex, Chesapeake, VA

Cost Element	Data Tracked During the Demonstration	Cost	
Capital Costs			
Design & Planning	- Personnel required and associated labor	Labor	$41,200
		Expense	$10,800
Well Installation	- Personnel required and associated labor - Mobilization costs - Drilling contractor cost	Labor	$12,100
		Expense	$48,200
O&M Costs			
Groundwater Amendments	- Personnel required and associated labor for groundwater ammendment activities - Mobilization costs - Costs for groundwater amendment chemicals (e.g., tracers, buffers) and equipment - JS666 culture costs - Cost for aeration devices and equipment	Labor	$49,900
		Expense	$28,100
Performance Monitoring Costs			
Baseline Characterization	- Personnel required and associated labor - Mobilization costs - Supplies and equipment for groundwater sampling - Sample shipment and laboratory analytical costs	Labor	$4,200
		Expense	$8,200
Performance Monitoring	- Personnel required and associated labor - Mobilization costs - Supplies and equipment for groundwater sampling - Sample shipment and laboratory analytical costs - Labor associated with data reporting	Labor	$58,100
		Expense	$54,100

Aquifer Geochemistry

- **Groundwater pH** - Relatively neutral pH (6.5 to 8) is required to provide optimal growth conditions for the JS666 culture. Sites where the groundwater pH is outside of this range may require chemical alteration of the groundwater (e.g., addition of a buffer) to achieve a desirable pH. The added costs for buffer, amendment equipment, and labor required to inject the buffer will increase capital and operational costs of the technology. Ultimately, it may be possible to adapt JS666 to lower pH through selection of low-pH-tolerant variants.

- **Presence of other organic constituents** - Co-presence of VC, TCE or 1,2-DCA can reduce the maximum degradation rate of cDCE. Thus the presence of co-contaminants may require additional bioaugmentation culture and longer timeframes for remediation, which would increase operational costs.

- **Dissolved oxygen** - JS666 has an absolute requirement for molecular oxygen but has been found to function at oxygen levels as low as 0.01 mg/L. Oxygen concentrations above 10 mg/L are inhibitory. Sites where groundwater DO levels are low may require additional amendments to increase groundwater DO to an ideal level (i.e., a minimum of 0.8 mg/L oxygen per 1 mg/L cDCE). The added costs for chemicals and/or oxygen delivery equipment will increase capital and operational costs of the technology.

Aquifer Geology and Hydrogeology

- **Hydraulic conductivity** - Microorganisms and other groundwater amendments may be more readily distributed in permeable media. Sites with a low hydraulic conductivity (K) will generally be more expensive because a greater number of injection points are required to treat a given area.

- **Geological heterogeneity** - High heterogeneity limits the uniform distribution of microorganisms and other groundwater amendments within the target treatment area. Thus treatment of sites with high heterogeneity will generally be more expensive as they may require a greater number of injection points or longer timeframes for remediation.

- **Depth of impacted aquifer** – Deep groundwater plumes will be more expensive to treat as they require deeper injection and monitoring wells, which are more expensive to install.

Bioaugmentation System Design

- **Well size, depth and number** - The cost of wells required to implement the technology is proportional to the depth of installation and number of wells required to treat a given area.

- **Cost of JS666 culture and other groundwater amendments** - The dose/cost of the JS666 culture is relative to the size of the treatment area. The frequency and dose of other groundwater amendments (e.g., oxygen, pH buffer) will also impact O&M costs.

- **Ability of the JS666 culture to migrate away from injection points** - The further the culture can be distributed from the injection points, the fewer injection points that are required to treat a given area. Fewer injection points will reduce the cost for well installation.

Available Infrastructure & Site Access

- **Available infrastructure** - The availability of infrastructure (e.g., existing groundwater injection or monitoring wells, storage buildings, and utilities) can reduce the cost of technology implementation.

- **Site Access** - Sites having limited access for equipment and personnel (e.g., difficult terrain, overhead obstructions, or treatment beneath a building) may incur higher costs when implementing the technology.

7.3 COST ANALYSIS

The cost model was developed for a template site with cDCE- impacted shallow groundwater. The specific site characteristics are similar to those observed at the test site used in the current technology demonstration and are presented in Table 7-2. A cost estimate was also prepared for a conventional pump and treat system to provide a point of comparison with the EISB approach using JS666. The cost model focused on treatment of a contaminated plume of groundwater. Specifically excluded from consideration are the costs of pre-remediation investigations (e.g., plume delineation, risk determination, and related needs), treatability studies, permitting, source zone treatment, and post remediation and decommissioning. Also excluded are costs for waste (e.g., soil cuttings and well development water) characterization and disposal.

The template site assumes a homogenous silty sand aquifer to a depth of 18 ft bgs with a hydraulic conductivity of 7 ft/d, a horizontal gradient of 0.007 ft/ft and an effective porosity of 0.25. These aquifer characteristics result in a groundwater seepage velocity of approximately 72 ft/yr. Depth to water is 4 ft bgs. The plume of cDCE-impacted groundwater extends along the direction of groundwater flow for 500 ft and is 200 ft in width. Concentrations of cDCE, TCE and VC in the plume are 1,000 µg/L, 475 µg/L, and 15 µg/L, respectively. Both alternatives were designed to achieve treatment to USEPA MCLs (70 µg/L, 5 µg/L, and 2 µg/L for cDCE, TCE and VC, respectively).

The EISB using JS666 approach assumes 40 direct push injection points and six 2-inch monitoring wells screened within the saturated zone. The injection point layout assumes 2 transects of 20 injection points each, staggered injection point placement, 10 ft spacing between injection points, and a radius of influence of 2.5 ft, thus creating a biobarrier that measures 200 ft

TABLE 7-2: Basis of Cost Analysis
Site 21, St. Julien's Creek Annex, Chesapeake, VA

Parameter	Unit	Quantity
Target Treament Area Dimensions & Hydrogeology		
Total Depth	ft bgs	18
Depth to Water	ft bgs	4
Saturated Thickness	ft	14
Width	ft	200
Length	ft	500
Effective Porosity	v/v	0.25
Hydraulic Conductivity	ft/d	7
Horizontal Gradient	ft/ft	0.007
Seepage Velocity	ft/yr	72
Geochemistry		
Average Groundwater cDCE Concentration	µg/L	1,000
Average Groundwater TCE Concentration	µg/L	475
Average Groundwater VC Concentration	µg/L	15
Treatment Parameters		
Duration of Pump and Treat	years	30
Duration of EISB	years	30
Discount Rate	%	2.7

Notes
v - volume
gal - gallon
ft bgs - feet below ground surface
ft - feet
ft/d - feet per day
ft/yr - feet per year
µg/L - micrograms per liter
% - percent

wide by 10 ft long (in the direction of groundwater flow). To facilitate the cost analysis, it was assumed that the groundwater pH and dissolved oxygen levels at the template site are suitable for growth of the JS666 culture and that no pH or buffer amendments are required. Assuming post-bioaugmentation degradation rates of -2.38/d, -2.23/d, and -2.55/d (estimated from laboratory microcosm tests; Geosyntec, GIT & Cornell University, 2008) for cDCE, TCE and VC, respectively, the residence times required for these compounds to be degraded to MCLs are approximately 1.1 days, 2 days, and 0.8 days, respectively, which are all considerably less than the estimated hydraulic residence time of 51 days for groundwater travelling through the biobarrier.

The pump and treat system assumes two groundwater extraction wells screened within the saturated zone and equipped with electrically-operated submersible pumps. The maximum total groundwater extraction rate is assumed to be 2 gpm. Extracted groundwater will be treated using granular activated carbon and then recharged into the shallow aquifer via an infiltration gallery.

Summaries of the costs for EISB using JS666 and the pump and treat alternatives are provided in Tables 7-3 and 7-4. The capital cost for the EISB using JS666 alternative, which includes installation of wells and bioaugmentation, is approximately $80K. The annual monitoring cost is estimated to be $29K per year. The capital cost for the pump and treat alternative is $264K, which is significantly higher than the capital cost for the EISB using JS666 alternative. The annual O&M costs of $56K per year are also higher than those of the EISB using JS666 alternative.

Life-cycle costs for the two technologies were calculated using Net Present Value (NPV) of future costs and assuming a 30 year remediation timeframe. O&M and long-term monitoring costs are discounted at a rate of 2.7% based on the real discount rate provided by the U.S. Federal Government Office of Management and Budget for 30-year notes and bonds (Office of Management and Budget, 2008).

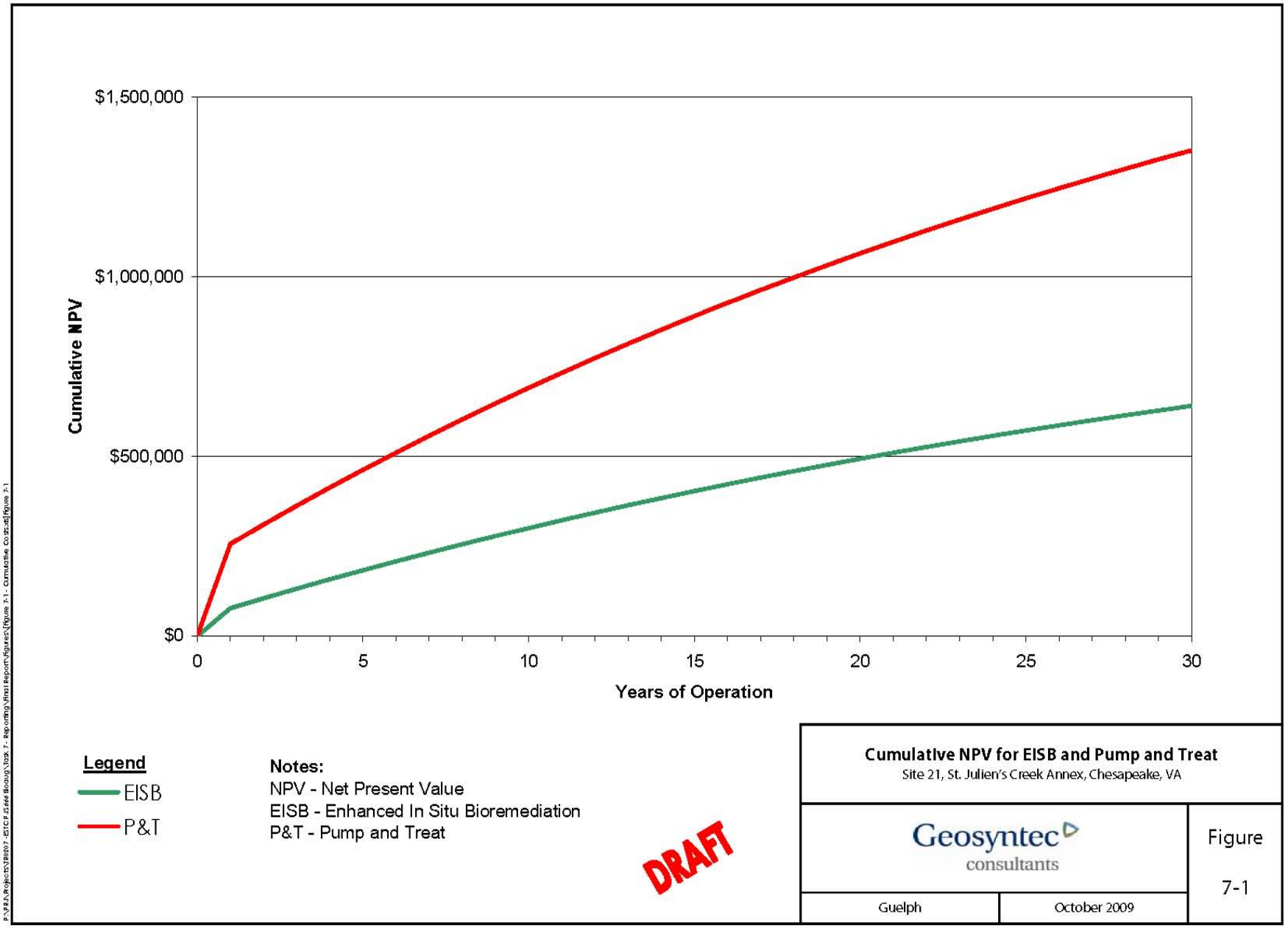
Figure 7-1 shows the cumulative NPV costs by year for the EISB using JS666 and pump-and-treat alternatives evaluated above. The total NPV cost for the EISB using JS666 alternative is estimated to be $641K, and the total cost of the remedy over 30 years is estimated to be $922K. The total NPV cost for the pump and treat alternative is estimated to be $1,352K, and the total cost of the remedy over 30 years is estimated to be $1,901K; both cost estimates are significantly higher than those for the EISB using JS666 alternative.

TABLE 7-3: Cost for EISB Using JS666
Site 21, St. Julien's Creek Annex, Chesapeake, VA

Task Description	Unit	Unit Cost	Quantity	Cost ($)	Cost ($) with 20% Contingency
Monitoring Well Drilling					
Six (6) 2-inch monitoring wells, installed to 18'. Mobilization, per diem, decontamination, drums included	ea	$1,321	6	$7,928	$9,514
Drilling Oversight (Staff Professional)	hr	$85	108	$9,180	$11,016
Travel, per diem	LS			$4,800	$5,760
Drilling Subtotal				$21,908	**$26,290**
JS666 Injection					
Forty (40) injection points. Mobilization, per diem included	ea	$500	40	$20,000	$24,000
JS666 culture	L	$250	78	$19,460	$23,352
JS666 injection (Staff Professional)	hr	$85	40	$3,400	$4,080
Travel, per diem	LS			$1,920	$2,304
First Year JS666 Injection Subtotal				$44,780	**$53,736**
Total Capital Costs (Incl. Contingency)					**$80,026**
Annual Long-term Monitoring Cost					
Performance monitoring (including sampling and analysis)	sample	$300	24	$7,200	$8,640
Reporting	LS			$15,000	$18,000
Annual Long-term Monitoring Cost Subtotal				$24,200	**$29,040**
Total Annual Long-term Monitoring Cost (Incl. Contingency)					**$29,040**

TABLE 7-4: Cost for Pump and Treat
Site 21, St. Julien's Creek Annex, Chesapeake, VA

Task Description	Unit	Unit Cost	Quantity	Cost ($)	Cost ($) with 20% Contingency
Extraction Well Drilling					
Installation of two (2) 4-inch extraction wells, installed to 18'.					
Mobilization, per diem, decontamination, drums included	ea	$3,200	2	$6,400	$7,680
Drilling Oversight (Staff Professional)	hr	$85	18	$1,530	$1,836
Travel, per diem	LS			$1,120	$1,344
Drilling Subtotal				$7,520	**$10,860**
Treatment System Construction and Startup					
Design, planning and procurement (Professional)	hr	$110	275	$30,250	$36,300
Piping, instrumentation and process control equipment	LS			$136,900	$164,280
Infiltration gallery	LS			$12,500	$15,000
Construction supervision/oversight (Staff Professional)	hr	$85	270	$22,950	$27,540
Startup Testing (Staff Professional, Technician)	hr	$85	27	$2,295	$2,754
Travel, per diem	LS			$6,080	$7,296
Treatment System Construction and Startup Subtotal				$180,725	**$253,170**
Total Capital Costs (Incl. Contingency)					**$264,030**
Annual O&M and Long-term Monitoring Cost					
Activated carbon changeout	ea	$543	14	$7,602	$9,122
Process monitoring and maintenance (Technician)	hr	$55	208	$11,440	$13,728
Performance monitoring (including sampling and analysis)	sample	$250	52	$13,000	$15,600
Reporting	LS			$15,000	$18,000
Annual O&M and Long-term Monitoring Cost Subtotal				$47,042	**$56,450**
Total Annual O&M and Long-term Monitoring Cost (Incl. Contingency)					**$56,450**


$1,500,000
$1,000,000
$500,000
$0
Cumulative NPV
0
5
10
15
20
25
30
Years of Operation
Legend
EISB
P&T
Notes:
NPV - Net Present Value
EISB - Enhanced In Situ Bioremediation
P&T - Pump and Treat
DRAFT
Cumulative NPV for EISB and Pump and Treat
Site 21, St. Julien's Creek Annex, Chesapeake, VA
Geosyntec consultants
Figure
7-1
Guelph
October 2009

8.0 IMPLEMENTATION ISSUES

This section provides information that will assist in future implementations of the technology. The following are four key issues related to implementation of the JS666 bioaugmentation technology:

- Permitting

  For this pilot test, only an injection notification letter was required by EPA Region 3. At full-scale, an underground injection control (UIC) permit may be required for the injection of bacteria and buffer amendments (if needed) and extraction and re-injection of contaminated groundwater.

- Buffer Addition

  If the pH of the groundwater is low, buffer addition will be required. In a passive system, like the one demonstrated here, one way to distribute the buffer is to extract groundwater, amend it with buffer and re-inject. This process can be time-consuming for lower permeability aquifers. Furthermore, because the buffer is soluble, it must be re-amended periodically. If a site has low pH, a recirculation system may prove more effective for metering in buffer solution and maintaining it in the treatment zone. However, recirculation systems typically have higher O&M costs than passive systems.

- Aeration

  If the ambient dissolved oxygen is not sufficient to support biodegradation, then aeration is required to raise groundwater oxygen levels. JS666 does not tolerate oxygen concentrations above 10 mg/L; thus, care must be taken not to achieve concentrations above this level. There are several options for introducing oxygen. Air biosparging or diffusive emitters (expensive at full scale) can be used. Other means to introduce oxygen include the use of peroxides (either solid or liquid). Because of the possibility of achieving greater than 10 mg/L of dissolved oxygen locally, these products would need to be added some distance upgradient from where JS666 was injected to permit consumption of dissolved oxygen to levels that JS666 can tolerate.

- Contaminant Inhibition

  JS666 can degrade cDCE metabolically and TCE and VC cometabolically. However, as the concentration of TCE increases, the rate of cDCE degradation decreases due to competitive inhibition. Therefore, JS666 will perform better when there are lower concentrations of TCE (<500 ug/L) in groundwater. To mitigate the effects of competitive inhibition due to high TCE concentrations to some extent, higher densities of JS666 can be employed.

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